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(71) Applicant *(for all designated States except US)*: UNIVERSITY OF VIRGINIA PATENT FOUNDATION [US/US]; Suite 1–110, 1224 West Main Street, Charlottesville, VA 22903 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BEACH, James, M. [–/US]; Charlottesville, VA (US). TIEDEMAN, James, S. [–/US]; Charlottesville, VA (US).

(74) Agent: SUFFREDINI, Brian, R.; University of Virginia Patent Foundation, Suite 1–110, 1224 West Main Street, Charlottesville, VA 22903 (US).

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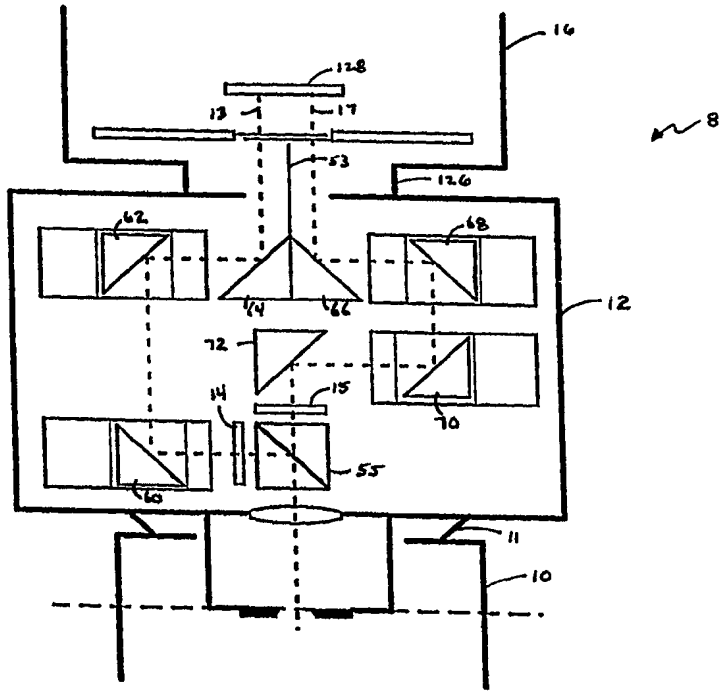
(57) Abstract

An apparatus for evaluating oxygen utilization in posterior pole tissue of an eye includes a fundus camera (10) capable of generating an intermediate image of blood vessels in an eye, a beam splitting assembly (12) comprising a first bandpass filter (14), a second bandpass filter (15), a beam splitting (55) capable of splitting the intermediate image generated by the fundus camera into first, second images, an electronic imaging device (16) capable of electronically recording the first, the second images after the first, the second images have passed through the first, and second bandpass filters. The first, and second bandpass filters have respective first, second wavelength chosen to optimize the blood oxygen saturation imaging capability of the electronic imaging device. A method for evaluating oxygen utilization in posterior pole tissue of an eye includes reflecting a light beam off of blood vessels in an eye to create an intermediate image, and splitting the intermediate image into first, second images which are filtered such that the first, the second images have respective first, and second wavelength which optimize the electronic recording with respect to blood oxygen saturation. Empirical relationships between oxygen saturation, and optical density are utilized to obtain oxygen saturation values for a given subject.



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IMAGING OCULAR VESSEL OXIMETER

CROSS-REFERENCE TO RELATED PROVISIONAL APPLICATION

The present application claims the benefit of the earlier filing date of U.S. Provisional Patent Application Serial No. 60/094,715, filed July 30, 1998, which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The present invention relates to an apparatus and method for evaluating oxygen utilization in the posterior pole tissue of the eye. More particularly, the present invention relates to a simple apparatus and method for measuring the hemoglobin oxygen saturation of retinal blood vessels using digital image analysis of reflectance at two specially chosen wavelengths and corrections for pigmentation and blood vessel diameter.

BACKGROUND OF THE INVENTION

The blood supply to the inner retina is well-regulated to maintain an adequate oxygen supply to the retinal tissue. Despite this circulation's key role in maintaining the viability of the inner retina, however, little is known about retinal oxygen consumption and how it changes in response to systemic disorders. It is believed that a poor oxygen supply to the inner retina may be the direct cause of many diseases, such as diabetic retinopathy, papallopathy, glaucoma, optic atrophy and macular degeneration, among others.

Diabetic retinopathy, for example, is a progressively damaging eye disease which effects approximately 14 million Americans. Diabetic retinopathy may create several retinal disorders, including swelling at the center of the retina, and reduction of retinal circulation. This leads to a loss of oxygen to the retina, which may cause the eye to grow

additional blood vessels that join with the gelatinous material in the center of the eye. It can lead to severe vision loss if left untreated, and is the leading cause of blindness for individuals under 40 years of age.

Like many eye diseases, diabetic retinopathy is preceded by early stage warning signs that can trigger severe damage if undetected. In diabetic retinopathy, such effects are referred to as background diabetic retinopathy ("BDR"), and include difficulty in dark adaptation, microaneurisms indicating weakening of blood vessels, and hemorrhaging of blood vessels into retinal tissues. Early detection of BDR is crucial because individuals at risk for retinopathy may arrest its progress through tight control of blood sugars and appropriate lifestyle alterations. However, eye damage is rarely detectable with an opthalmoscope during the first five years a patient experiences elevated blood sugars. Thus, there is a need for a technique for the early detection of diabetic retinopathy, as well as other eye diseases such as papallopathy, galucoma and macular degeneration.

Researchers have been attempting to address this deficiency in information concerning ocular oxygen consumption and how it changes in response to disease or other challenges since the 1960s, and have reported noninvasive optical methods for determining the blood oxygen saturation ("O_2SAT") in large retinal vessels in humans and animals. These methods depend on quantifying differences between oxyhemoglobin and deoxyhemoglobin light absorption spectra at different wavelengths.

In particular, a retinal oximetry technique developed by Pittman, et al. uses three wavelengths transmitted through vessels in a hamster cheek pouch. See Pittman, et al., "A New Method For The Measurement Of Percent Oxyhemoglobin," J. Appl. Physiol. 38 (1975) at 315-20; Pittman, et al., "Measurement Of Percent Oxyhemoglobin In The

Microvasculature," J. Appl. Physiol. 38 (1975) 321-27. By measuring the optical density ("OD") of blood within vessels at three closely-spaced wavelengths, two isosbestic and one oxygen-sensitive, Pittman, et al. derived a relationship between the three ODs and O_2SAT independent of external calibration. From measured light intensities at two isosbestic wavelengths, they corrected for light lost from the measuring beam due to scattering by red blood cells, and determined the light absorption of the blood column. The transmission method used by Pittman, et al. bears no resemblance to the reflectance method of the present invention.

Because transmission OD measurements are not feasible in the retinal circulation, Delori, et al. adapted the oximetry method of Pittman, et al. to record reflectance measurements from the ocular fundus. See Delori, et al., "Oxygen Saturation Of Retinal Veins In Optic Atrophy," Invest. Opthamol. Vis. Sci. 27 (ARVO Suppl.) (1986) at 221; Delori, "Noninvasive Technique For Oximetry Of Blood In Retinal Vessels," Appl. Optics 27 (1988) at 1113-25. The method of Delori, et al. employs a mechanical scanner in a modified fundus camera equipped with a photomultiplier detector to monitor sequentially the light reflected inside and outside vessels at three wavelengths. Using the theoretical relationship between OD and light absorption of whole blood, Delori, et al. attempted to avoid the need for external calibration. Yet the Delori, et al. approach requires instrumentation to continuously scan regions of the fundus at successive wavelengths obtained from a tungsten lamp. Unlike the apparatus of the present invention, such instrumentation is complex and difficult to control due to eye motion, and the result has not been widely used. In addition, in contrast to the approach of the present invention, Delori, et al. eschewed an empirical approach, attempting instead to measure hemoglobin O_2SAT using a biophysical model with

evaluation of critical variables required to obtain a result. Thus, the approach of Delori, et al. bears no resemblance to that of the present invention.

Hickam, et al. and Laing, et al., investigated the use of photographic densitometry of large retinal vessels in vivo to monitor arterial and venous retinal blood O_2SAT. See Hickam, et al., "Studies Of The Retinal Circulation In Man: Observations On Vessel Diameter, Areteriovenous Oxygen Difference, And Mean Circulation Time," Circulation 33 (1966) at 302-16; Hickham, et al., "A Study Of Retinal Venous Blood Oxygen Saturation In Human Subjects By Photographic means," Circulation 27 (1963) at 375-83; Laing, et al., "Photographic Measurements Of Retinal Blood Oxygen Saturation: Falling Saturation Rabbit Experiments," Invest. Opthamol. 14 (1975) at 606-10. Both concluded that accurate measurements could be made using the ratio of vessel ODs at two different wavelengths, but significant disadvantages are inherent in their approaches. For example, the use of photographic imaging media, i.e. black and white film, precluded Hickam, et al. and Laing, et al. from comparing images easily and with a high degree of control and reproducibility. Such comparisons are laborious, requiring densitometric analysis of photographic negatives under highly controlled conditions, and are inherently limited by the nonlinearity and variable reproducibility of photographic film. The method of Hickam, et al. also employed wavelengths far apart from one another (640 nm and 510 nm), inviting error due to the differences in light scatter efficiency in the retinal tissue at such wavelengths. In addition, the methods of Hickam, et al. and Laing, et al. depended upon external calibration by means of independent arterial O_2SAT measurement. Thus, the approaches of Hickham, et al. and Laing, et al. are not amenable to providing a large volume of analyses such as would be required, for example, in a clinical setting. Indeed, until the present invention, no

oximetry approach suggested was capable of providing a level of simplicity, speed, sensitivity and reproducibility that would be appropriate for such a setting.

It is therefore an object of the present invention to provide an apparatus and method for evaluating blood oxygen saturation in vessels of the eye that are more simple and controllable, and that provide more reproducible results, than those previously proposed.

It is another object of the present invention to provide an apparatus and method for evaluating blood oxygen saturation in vessels of the eye that avoid the use of photographic film.

It is also an object of the present invention to provide an apparatus and method for evaluating blood oxygen saturation in vessels of the eye that are based upon reflectance principles and utilize wavelengths that are more closely matched than those previously proposed, to reduce the effect of light scattering in tissue.

It is another object of the present invention to provide a method for empirically determining the variables required to quantify blood oxygen saturation in vessels of the eye.

It is another object of the present invention to provide a method for quantifying blood oxygen saturation in vessels of the eye without the need for an external calibration accompanying the measurement.

It is another object of the present invention to provide a method for quantifying blood oxygen saturation in vessels of the eye that is not adversely affected by eye motion.

It is another object of the present invention to provide a method for quantifying blood oxygen saturation in vessels of the eye that corrects for the effects of pigmentation.

It is another object of the present invention to provide a method for quantifying blood oxygen saturation in vessels of the eye that corrects for the effects of vessel diameter.

It is another object of the present invention to provide an apparatus and method for automatically quantifying blood oxygen saturation in vessels of the eye.

It is another object of the present invention to provide an apparatus and method for quantifying blood oxygen saturation in vessels of the eye that is appropriate for use in a clinical setting.

SUMMARY OF THE INVENTION

In accordance with the principles of the present invention, an apparatus for evaluating oxygen utilization in posterior pole tissue of an eye includes a fundus camera capable of generating an intermediate image of blood vessels in an eye, and a beam splitter assembly comprising a beam splitter, a first bandpass filter and a second bandpass filter. The beam splitter is capable of splitting the intermediate image generated by the fundus camera into first and second images. Preferably, a baffle is disposed between the first image and the second image proximal to the location where the light exits the beam splitter assembly, to block stray light between the first and second images emerging from beam splitter assembly. The apparatus of the present invention further includes an electronic imaging device capable of electronically recording the first and second images after the first and second images have passed through the respective first and second bandpass filters. The electronic imaging device may be of any known type, such as a digital imaging device, and preferably is a charge-coupled device with a digital read-out. Although the main components of the apparatus of the present invention typically comprise separate but connected units, the fundus

camera, beam splitter and electronic imaging device may comprise an integral unit.

After the intermediate image is split by the beam splitter, the first and second images are passed through the first and second bandpass filters, respectively. The first bandpass filter has a first wavelength and the second bandpass filter has a second wavelength, the first and second wavelengths being chosen to optimize the imaging capability of the electronic imaging device with respect to O_2SAT. Although other wavelengths may be chosen, as will be described in greater detail herein, the wavelength of the first bandpass filter preferably is chosen to be an oxygen-sensitive wavelength of 600 nm ± 2.5 nm, and the second wavelength of the second bandpass filter preferably is chosen as 569 nm ± 2.5 nm, 586 nm ± 2.5 nm or 558 nm ± 2.5 nm.

In one embodiment of the apparatus of the present invention, the fundus camera has an imaging plane external to the body of the fundus camera. However, other fundus cameras may be used which have an imaging plane internal to the body of the fundus camera. In the embodiment of the present invention in which the imaging plane is formed external to the body of the fundus camera, the fundus camera may comprise a filter wheel fitted with a -6 diopter lens for this purpose.

The beam splitter assembly may have a plurality of front surface mirrors to position the first and second images relative to each other, and a cover may be disposed over the beam splitter assembly to protect it from contamination. Preferably, one or more of the plurality of front surface mirrors is mounted so as to be adjustable, and the beam splitter assembly comprises means for mounting the plurality of front surface mirrors in the beam splitter assembly. The means for mounting the plurality of front surface mirrors in the beam splitter assembly may include a base plate. The base plate may have a first side portion with a

first plurality of rails, and a second side portion with a second plurality of rails. The first and second pluralities of rails may be used to support first and second pluralities of mirror mounts, respectively. A first portion of the front surface mirrors may be attached to the first plurality of mirror mounts and a second portion of front surface mirrors may be attached to the second plurality of mirror mounts. A first drive assembly may be selectively attached at least one of the first plurality of mirror mounts, wherein movement of the first drive assembly may result in movement of at least one of the front surface mirrors. A second drive assembly may be selectively attached to at least one of the second plurality of mirror mounts, wherein movement of the second drive assembly may result in movement of at least one of the front surface mirrors. The first drive assembly may comprise a first micrometer and the second drive assembly may comprise a second micrometer.

The present invention is also drawn to a method for evaluating oxygen utilization in posterior pole tissue of an eye. An embodiment of the apparatus of the present invention, or another apparatus, may be used to perform this method. The method includes reflecting a light beam off of blood vessels in an eye to create an intermediate image of the blood vessels, splitting the intermediate image into a first image and a second image, and filtering the first and second images such that the first image has a first wavelength and the second image has a second wavelength. The method further comprises producing an electronic recording of the filtered first and second images. In this method, the first wavelength of the first image and the second wavelength of the second image are chosen to optimize the electronic recording with respect to O_2SAT.

Once the first and second images have been recorded, average reflected light intensity inside and outside of an imaged blood vessel ("I_{in}" and "I_{out}", respectively) may be

determined from the first and second images of the blood vessel by scanning the first image and the second image. The path of minimum reflection inside the blood vessel may then be identified, and a value of I_{in} along the path of minimum reflection determined. Next, a path of extravascular reflection in the image at a fixed distance from the path of minimum reflection inside said first of said blood vessels is identified, and a value of I_{out} along the path of extravascular reflection determined. This is done for both the first image and the second image. Once the I values are determined, a value for O_2SAT may be found.

In accordance with the present invention, the I values, along with other data determined from the electronically recorded first and second images, are utilized along with empirical relationships between venous and arterial O_2SAT ("O_2SAT_{vein}" and "O_2SAT_{artery}," respectively) and OD to obtain a value of O_2SAT_{vein} for a given subject. Values of O_2SAT_{vein} and/or O_2SAT_{artery} may also be determined for a given subject at different times after an intervention, to detect a change in such values. Although other methods will be appreciated as obvious variations of those of the present description, three methods are described for determining the values of the variables required for determining O_2SAT_{vein} from such empirical relationships: the direct reflectance method, the pigmentation correction method, and the intravascular reflectance method.

The direct reflectance method comprises determining values of arterial optical density ratio ("ODR_{artery}") for a subject using data contained in the electronic recording of the first and second images, and comparing the ODR_{artery} values to values of systemic O_2SAT corresponding therewith (obtained using a pulse oximeter or other conventional instrument for obtaining values of systemic O_2SAT) to determine oxygen sensitivity ("OS"), the slope of the ODR_{artery} vs. systemic O_2SAT curve. Once a value for OS is determined, a simple

equation may be employed to obtain a value for O_2SAT_{vessel} at any time for the subject.

The pigmentation correction method is used to reduce the influences of pigmentation on calculated values of O_2SAT_{vein}. Under this method, unless previously determined, the values of the variables required for determining O_2SAT_{vessel} must first be obtained using the pigmentation correction method. This comprises estimating, for a plurality of subjects, the reflectance at the second chosen wavelength, where pigment light absorption is strong, using the reflectance at the first chosen wavelength where pigment light absorption is significantly less than that at the second wavelength. The corrected ODR_{artery} ("$ODR_{cor,artery}$") values are compared to values of systemic O_2SAT corresponding therewith to determine a value for pigmentation corrected OS. Once a value for pigmentation corrected OS is determined, a simple equation may be employed to obtain a value for O_2SAT_{cor} for an artery or vein of any subject at any time.

The intravascular reflectance method permits the determination of O_2SAT_{vein} or O_2SAT_{artery} values independent of pigmentation effects. Like the pigmentation correction method, unless previously determined, the values of the variables required for determining O_2SAT_{vessel} must first be obtained using the intravascular reflectance method. This method effects such a calibration by using values of ODR_{artery} determined for a plurality of subjects assuming that each subject's fundus is unpigmented. The ODR_{artery} values are then compared with values of systemic O_2SAT corresponding therewith to determine OS, and an O_2SAT_{vessel} value may be calculated independent of pigmentation for any subject at any time.

The O_2SAT_{vessel} results obtained using the pigmentation correction and intravascular reflectance methods may also be corrected for vessel size. This is done by determining, for the subjects from which data is collected, diameters of the arteries being

measured, and comparing ODR_{artery} to the artery diameters to determine a value of vessel diameter sensitivity ("C").

The variables determined using the direct reflectance method are accurate for the subject from which the data used was obtained, but may not be as accurate for other subjects. However, if the values of the variables determined using the calibrations of the pigmentation correction and intravascular reflectance methods are determined using a statistically relevant group of subjects having varying pigmentations, such values should be useful to determine O_2SAT_{vessel} for any subject. In a clinical or other setting, the determination of O_2SAT_{vessel} (or a change in O_2SAT_{vein} or O_2SAT_{artery} after an intervention) in accordance with the method of the present invention, and preferably using the imaging ocular oximeter of the present invention, is useful to diagnose eye diseases, including such diseases as diabetic retinopathy, papallopathy, glaucoma, retinal vein occlusion, optic atrophy and macular degeneration. Preferably, the determination of O_2SAT values is automated using a computer, such that the electronically recorded first and second images are scanned, analyzed and O_2SAT determined automatically.

The foregoing and other features, objects and advantages of the present invention will be apparent from the following detailed description, taken in connection with the accompanying figures, the scope of the invention being set forth in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic illustration of one embodiment of the imaging ocular vessel oximeter of the present invention;

FIG. 2 is a schematic illustration of a commercially available fundus camera which may be used in the imaging ocular vessel oximeter of the present invention;

FIG. 3a is an orthographic top view, scale 1" = 2", of one embodiment of an assembly for mounting the components of a beam splitter assembly in accordance with the present invention;

FIG. 3b is an orthographic side view, scale 1" = 2", of the assembly of FIG. 3a;

FIG. 3c is another orthographic side view, scale 1" = 2", of the assembly of FIG. 3a;

FIG. 4a is an orthographic top view, scale 3/4" = 1", of the center portion of the base plate shown in the assembly of FIGS. 3a-3c;

FIG. 4b is an orthographic front view, scale 3/4" = 1", of the center portion of the base plate shown in FIGS. 3a-3c;

FIG. 4c is an orthographic side view, scale 3/4" = 1", of the center portion of the base plate shown in FIGS. 3a-3c;

FIG. 5a is an orthographic top view, scale 1" = 2", of the side portions of the base plate shown in the assembly of FIGS. 3a-3c;

FIG. 5b is an orthographic side view, scale 1" = 2", of the side portions of the base plate shown in FIGS. 3a-3c;

FIG. 5c is another orthographic side view, scale 1" = 2", of the side portions of the base plate shown in FIGS. 3a-3c;

FIG. 5d is an orthographic front view, scale 1" = 2", of the side portions of the base plate shown in FIGS. 3a-3c;

FIG. 6a is an orthographic top view, full scale, of the mirror mount shown in the assembly of FIGS. 3a-3c;

FIG. 6b is an orthographic side view, full scale, of the mirror mount shown in

FIGS. 3a-3c;

FIG. 7a is an orthographic top view, full scale, of the first drive block shown in the assembly of FIGS. 3a-3c;

FIG. 7b is an orthographic side view, full scale, of the first drive block shown in FIGS. 3a-3c;

FIG. 8a is an orthographic top view, full scale, of the second drive block shown in the assembly of FIGS. 3a-3c;

FIG. 8b is an orthographic side view, full scale, of the second drive block shown in FIGS. 3a-3c;

FIG. 9a is an orthographic top view, scale 1" = 5", of one embodiment of a cover for use with beam splitter assembly of FIGS. 3a-3c;

FIG. 9b is an orthographic front view, scale 1" = 5", of the cover of FIG. 9a;

FIG. 9c is an orthographic side view, scale 1" = 5", of the cover of FIG. 9a;

FIG. 10 is a side-by-side composite of two simultaneous, digitally recorded images of retinal vessels near the optic disk, taken in accordance with the present invention using bandpass filters having center wavelengths 600 nm and 569 nm;

FIG. 11 is a side-by-side composite of two computer-generated scans of simultaneous retinal images taken using bandpass filters having center wavelengths 600 nm and 569 nm;

FIG. 12 is a schematic illustration of a breathing circuit used to collect data from subjects in accordance with the present invention;

FIG. 13 is a plot showing the relationship between ODR, calculated using the direct reflectance method of the present invention, and O_2SAT;

FIG. 14 is a plot showing the relationship between ODR_{cor}, calculated using the pigmentation correction method of the present invention, and O_2SAT;

FIG. 15 is a plot showing the relationship between ODR_{cor} calculated at 100% O_2SAT_{artery} using the pigmentation correction method of the present invention, and vessel diameter (upper panel), and a plot showing the relationship between regression slope, calculated using the intravascular reflectance method of the present invention, and vessel diameter (lower panel); and

FIG. 16 is a plot showing the relationship between ODR_{irm}, calculated using the intravascular reflectance method of the present invention, and systemic O_2SAT_{artery}.

DETAILED DESCRIPTION OF THE INVENTION

The imaging ocular vessel oximeter of the present invention, and the method of the present invention for measuring O_2SAT and changes in O_2SAT, will now be described with reference to the embodiments illustrated in FIGS. 1 through 15.

As exemplified in the embodiment of FIG. 1, an imaging ocular vessel oximeter 8 made in accordance with the principles of the present invention includes a fundus camera 10, a beam splitter assembly 12, bandpass filters 14 and 15 and an imaging device 16. The design of the fundus camera shown in the embodiment of FIG. 1 and illustrated schematically in FIG. 2 is based upon a Kowa RC/W fundus camera, manufactured by Kowa Company Ltd. of Tokyo, Japan, modified as will be described. The Kowa RC/W fundus camera as provided by the manufacturer has a body 19 and an imaging plane external to the camera, beyond exit aperture 18. As is preferable in performing the present invention, fundus camera 10 shown in FIG. 2 was formed by fitting the Kowa RC/W with a -6 diopter lens 20 in a filter wheel 46 to form an intermediate image at an intermediate image plane 26 just

before exit aperture 18. A slit mask 28 is positioned at intermediate image plane 26 slightly off the optical axis to avoid central light artifacts produced by the fundus camera.

It will be appreciated that, although the modifications to fundus camera 10 described above are useful, they are not essential to the present invention. In addition, fundus camera 10 may be any conventional or specially designed fundus camera.

In fundus camera 10 of FIG. 2, an internal xenon flash 30 is provided to illuminate a patient's retina. Flash energy is supplied in this embodiment by a Model 404 external strobe power supply (not shown), manufactured by Norman Co. of Burbank, California, at a setting of 200 Joules, providing a retinal exposure per flash of approximately 100 mJ cm^{-2} (40° field). Flashes are synchronized with the operation of imaging device 16 in the conventional manner. Wavelengths above and below the wavelengths to be recorded are eliminated with a broadband illumination filter 32 which, in this embodiment, is a 580 nm center, 60 nm half-width filter. The light from the flash then passes through a condensing lens 34, is redirected by mirrors 36, 38, and passes through an opthalmological lens 40 to the retina of a patient's eye. Reflected light returns back through opthalmological lens 40, through a mirror aperture 42, through a negative lens 44 contained in filter wheel 46, and through a variable focus objective lens 48, producing the intermediate image at slit mask 28 which projects past the backplate 50 of fundus camera 10. A second positive lens 52, a 2-element achromatic converging lens, is placed after slit mask 28 and before beam splitter assembly 12 to refocus the images at the image sensor. In the embodiment of FIG. 2, second positive lens 52 is manufactured by Edmund Scientific of Barrington, New Jersey.

In the embodiment of FIG. 1, beam splitter assembly 12 is mounted to fundus camera 10 by means of a mounting assembly 11 originally provided with the fundus camera

for mounting a film pack. In other embodiments, however, beam splitter assembly 12 may be mounted another way or be manufactured as an integral unit with fundus camera 10 and/or with imaging device 16. As shown in the embodiment of FIG. 1, beam splitter assembly 12 is used to separate the light forming the intermediate image into first and second image paths of identical lengths, and ultimately into first and second laterally displaced, simultaneous images 13 and 17 at imaging device 16. Although other beam splitting devices may be used in connection with the present invention, beam splitter assembly 12 of the embodiment illustrated in FIGS. 1 and 2 comprises a dichroic mirror 55, first bandpass filter 14 and a second bandpass filter 15 placed in each optical path emanating from dichroic mirror 55. The beam splitter assembly 12 represented in FIG. 1 is manufactured by Optical Insights of Tucson, Arizona. The dichroic mirror 55 represented in FIG. 1, a dichroic filter cube with a 585 nm transition beam splitter (585DRLP02) mounted in an XF40 microscope filter cube, is manufactured by Omega Optical Co. of Brattleboro, New Hampshire.

Significantly, under the present invention, the wavelengths of first and second bandpass filters 14 and 15 should be chosen to optimize the O_2SAT signal, and therefore the imaging capability, of imaging device 16 with respect to O_2SAT. In particular, first bandpass filter 14 should be chosen to have a wavelength that yields sufficient contrast to enable automatic computer vessel tracking, and the greatest degree of oxygen sensitivity. The wavelength of second bandpass filter 15 should be chosen to be either completely insensitive to oxygen or highly sensitive in the opposite direction to the sensitivity of the oxygen-sensitive wavelength of first bandpass filter 14, to maximize the sensitivity of the measurement. Use of an insensitive second wavelength for second bandpass filter 15 has the advantage of permitting the system noise to be identified. For the imaging technique

represented by the embodiment of FIGS. 1 through 9, a 600 nm center wavelength (600 nm ± 2.5 nm passband) was chosen for first bandpass filter 14 (light absorption of hemoglobin and deoxyhemoglobin at that wavelength differ by a large factor – approximately a factor of 4), and a 569 nm center wavelength (569 nm ± 2.5 nm passband) was chosen for second bandpass filter 15 (it is an isosbestic wavelength for hemoglobin and oxyhemoglobin). The 600 nm and 569 nm bandpass filters used in connection with the embodiment of FIGS. 1 through 9 are manufactured by Omega Optical Co., and are side mounted, 5 nm halfwidth interference bandpass filters blocked for UV and IR out to 1100 nm.

In light of the foregoing discussion, it will be appreciated that another oxygen-insensitive or oxygen-sensitive wavelength could have been chosen for second bandpass filter 15. For example, a 586 nm ± 2.5 nm wavelength could have been used, which, when paired with a 600 nm wavelength bandpass filter, would have the additional benefit of further decreasing the light scattering effect of the tissue because the two wavelengths are more closely matched. In addition, a highly sensitive wavelength such as 558 nm ± 2.5 nm could have been chosen for second bandpass filter 15, which would provide greater sensitivity when paired with a 600 nm wavelength bandpass filter because the change in light absorption would move in oppositive directions for any given change in O_2SAT.

Figure 10 shows the effect of differing oxyhemoglobin content on reflected light from retinal vessels near the optic disk. Reflected light intensities from arteries A and veins V at the isosbestic wavelength (569 nm) (shown in second image 17 of FIG. 10) are comparable, both appearing dark, whereas at 600 nm (shown in first image 13 of FIG. 10) the high percentage of oxyhemoglobin increases the reflectance of the artery A compared to the vein V, making arteries A appear lighter than veins V.

In the embodiment of FIG. 1, beam splitter assembly 12 also includes front surface mirrors 60, 62, 64, 66, 68, 70 and 72, which are used to effect a lateral displacement of first and second images 13 and 17. Although other mirrors may be used to equal effect, the 15 mm base, TechSpec right angle mirror with enhanced aluminum coatings manufactured by Edmund Scientific Co. of Brunswick, New Jersey, has been found to be acceptable for use in connection with the present invention. The positions of front surface mirrors 60 through 72 relative to first image 13 and second image 15 preferably are adjustable by any conventional means for adjustment, such as screws, bolts or other fasteners or other devices known in the art that would be useful for this purpose. One design for selectively fixing and adjusting the positions of front surface mirrors 60 through 72 is illustrated in FIGS. 3-8. As shown in FIG. 3, mounting assembly 74 may be used to secure front surface mirrors 60 through 72 in place, and selectively permit adjustment of their positions. Mounting assembly 74 includes a base plate 76, having a center portion 75, illustrated in FIG. 4, to which front surface mirrors 64, 66 and 72, dichroic mirror 55, and bandpass filters 14 and 15 are mounted to permit their 2° rotational adjustment about the optic axis. Base plate 76 further includes side portions 77, illustrated in FIG. 5, to which front surface mirrors 60, 62, 68 and 70 are attached. As shown in FIG. 5, side portions 77 of base plate 76 have rails 78, 80, 82 and 84 on which mirror mounts 86, 88, 90 and 92 are disposed. One configuration for mirror mounts 86, 88, 90 and 92 is illustrated in FIG. 6 in isolation, and in FIG. 3 as contemplated for use in mounting assembly 74. In the embodiment of FIG. 3, for example, front surface mirror 68 is mounted to mirror mount 86 by a common vinyl doping compound, epoxy or another adhesive or attachment means. Mirror mount 86 is mounted on rail 82 and nominally held fixed in position by a nylon-tipped set screw (not shown) disposed in one of holes 94 in base plate 76.

If desired, the set screw may be loosened, and the position of mirror mount 86 and front surface mirror 68 selectively adjusted along rail 82 by means of a first drive assembly 95. Such adjustability permits the overall image magnification to be changed, and the first and second images 13 and 17 to be aligned.

In the embodiment of FIG. 3, first drive assembly 95 includes a first drive block 96 and a first micrometer assembly 98. As can be seen from FIGS. 3 and 7, first drive block 96 has a first screw bore 98 and a second screw bore 100, matching screw bore 102 on mirror mount 86 and screw bore 104 on mirror mount 88. Screws are secured in these cooperating adjustment points, such that set screws through mirror mounts 86 and 88 may be used to individually, selectively and rigidly connect mirror mounts 86 and 88 to first drive block 96. First micrometer assembly 98 has a micrometer shaft 106 stabilized by a shaft support block 108 and aligned parallel to rails 82 and 84, and is designed to fit movably within a drive bore 110 of first drive block 96. Drive bore 110 is intersected by a threaded set screw bore 112 such that when micrometer shaft 106 is positioned within drive bore 110, a set screw may be used to fix micrometer shaft 106 in place. Once this is accomplished, movement of micrometer shaft 106 will effect movement of micrometer shaft 106 and one or both of first and second mirror mounts 86 and 88, depending upon whether one or both of the set screws of mirror mounts 86 and 88 have been tightened. Movement of first and second mirror mounts 86 and 88 effects movement of front surface mirrors 68 and 70 along rails 82 and 84, respectively. Movement of one or both front surface mirrors 68 and 70 changes the path length through beam splitter assembly 12, causing a change in magnification. Small displacements of one of front surface mirrors 68 or 70 with respect to the second of such mirrors cause the associated image to move laterally relative to the other image.

As illustrated in FIGS. 1 and 8, a similar arrangement exists for the positioning and selective adjustment of mirror mounts 90 and 92 and front surface mirrors 60 and 62 using rails 78 and 80 and a second drive assembly 114, including a second drive block 116 and a second micrometer assembly 118.

Where the design for selectively fixing and adjusting the positions of front surface mirrors 60 through 72 illustrated in FIGS. 3-8 is used, a cover 120 as illustrated in FIG. 9 may be used to protect beam splitter assembly 12 from contamination, and to ensure that excess light is not introduced. Cover 120 includes openings 122 and 124 for permitting micrometer shafts and set screws to extend into beam splitter assembly 12 and be externally adjusted. Openings 122 and 124 may be insulated using gaskets, diaphragms, baffles or other protective devices to preclude excess light from entering the assembly. Similarly, light-absorbing surface coatings may be used inside the assembly to eliminate any excess noise that would otherwise be created.

As illustrated in FIG. 1, an opaque light baffle 53 is used to block stray light between first image 13 and second image 17 emerging from beam splitter assembly 12 and to produce a line of dark pixels separating simultaneous first and second images 13 and 17 as recorded by imaging device 16 (i.e., line "L" in FIG. 10). Opaque light baffle 53 may be constructed of any suitable material that is generally opaque and nonreflective, and in the present embodiment is made of aluminum covered by electrical insulating tape and further blackened using flat black paint (not shown).

After leaving beam splitter assembly 12, first and second images 13 and 17 are recorded by imaging device 16, which is mounted to beam splitter assembly 12 using the imaging device mounting bracket 126 provided with the imaging device used. Imaging

device 16 may comprise any high-quality custom or commercially available electronic imaging system. In the embodiment of FIGS. 1 and 2, imaging device 16 is a Model OMA camera, manufactured by EG&G Park of Princeton, New Jersey. The Model OMA camera is an 18 bit air-cooled digital camera equipped with a 1024 x1024 pixel charge-coupled device detector 128. Where another type of imaging system is used for imaging device 16, the resolution preferably will be 12 bit or greater. When a EG&G Park Model OMA imaging device is used as imaging device 16, it may be controlled using the manufacturer's "HIDRIS" software or its equivalent.

The imaging retinal oximeter 8 of the embodiment shown in FIGS. 1 through 9 is inexpensive, compact and easy to use, and is appropriate for use in clinical and other settings. As persons skilled in the art will realize, however, FIGS. 1 though 9 illustrate only one potential embodiment of the imaging ocular vessel oximeter 8 of the present invention, and other obvious variations of the apparatus are contemplated. For example, as indicated previously, virtually any commercially available fundus camera could be used for fundus camera 10, and the remaining components of the system adjusted accordingly. In addition, imaging device 16 may comprise two separate imaging systems, both preferably being digital systems, each such system being positioned directly after first and second bandpass filters 14 and 15, eliminating the need and cost of front surface mirrors 60 through 72 and mounting assembly 74. First and second bandpass filters 14 and 15 could also be selected to have an oxygen-sensitive wavelength other than 600 nm, and an oxygen-insensitive wavelength other than 569 nm, as previously discussed.

In operation, the image acquisition step of the present invention is complete once first and second images 13 and 17 of a patient's retinal blood vessels are obtained using

imaging device 16. The image acquisition step is followed by a scanning step in which a search algorithm embedded in a computer software program is used to scan first and second images 13 and 17 and obtain I values from identical vessel segments in first and second images 13 and 17. In the computer generated scans of FIG. 11, first image 13 is a scan of a 600 nm image, and second image 17 is a scan of a 569 nm image. The search algorithm tracks the path 130 of the minimum reflection inside vessels V to determine a value of average reflected light intensity I_{in} within the vessel V and the path 132 of the extravascular reflection at a fixed distance from the minimum reflection to determine a value of average reflected light intensity I_{out} outside the vessel V, in both first and second images 13 and 17. To begin a scan and identify the path of minimum reflection, the initial x-y coordinate of the vessel segment is identified in second image 17 (the 569 nm image), where vessel contrast is greater than in first image 13 (the 600 nm image), and x- and y- offsets to the same anatomic point on first image 13 are determined. If light reflex artifacts are present in the center of the vessel, the starting point is chosen to lie to the side of the reflex.

Typically, between 30 and 120 rows or columns of elements in second image 17 are scanned, depending upon the orientation of the vessel, to establish the path of the minimum reflected light intensity along the vessel. Edges of the blood column may be identified using gradient detection filters (Sobel operators) embedded in the computer code. The edge is identified at pixels for which the sum of filter responses peaks. Accordingly, this procedure will not mistake smaller light gradients of the central vessel reflection for the edge of the blood column. Minimum intensity inside the vessel may then be found by scanning between the edges. It will be appreciated that in arteries, it may be necessary to use only the minimum-valued element on each line, whereas in veins, larger diameter and smaller central

reflexes may allow averaging minimum image intensities within a neighborhood of points. The final measurement of light reflection is obtained from the average of minima along the vessel segment. Extravascular light reflection may be determined from pixels at a fixed distance outside of vessels. It should be noted that the algorithm used in the present invention may be designed to average from both sides, or from only one side of the vessel or artery if strong reflections from nerve fibers are present on one side.

Similar measurements are obtained from first image 13 by adding the x- and y-offsets to the vessel path determined from second image 17. Contrast in the first image also aids in correctly scanning the vessel path. Similarly, dark pixel values from an area of the charge-coupled device detector 128 outside the fundus image (i.e. the dark line denoted as "L" in FIG. 10) are subtracted from measured intensities.

Following the scanning step, the acquired data may be used to calculate O_2SAT_{vessel}. This may be done simply and automatically, preferably using a computer program. Before a subject's hemoglobin saturation level can be quantified using this system, however, empirical values required for such quantification must be calculated, preferably using data acquired in a statistically relevant study of a wide range of individuals such that the values will be relevant for any subject for which hemoglobin oxygen saturation is to be measured. Significantly, under two approaches of the method of the present invention, such an initial calibration need only be done once in order to measure O_2SAT_{vessel} for any of an unlimited number and variety of individuals (although separate calibrations would likely be required for humans and animals).

Although other approaches will be appreciated from the present description which are mere extensions or obvious variants of the methods presented herein, three

approaches have been identified as potentially useful for performing the requisite initial calibration and determining values of O_2SAT_{vessel}. These approaches are denoted herein as the "direct reflectance method," the "pigmentation correction method," and the "intravascular reflectance method." For purposes of clarity, the following discussion of these approaches is written in the context of an apparatus and method utilizing a first bandpass filter 14 of 600 nm and a second bandpass filter 15 of 569 nm, although filters of other wavelengths are contemplated as previously explained.

Direct Reflectance Method

In accordance with the direct reflectance method, a direct calculation of the apparent vessel optical densities OD_{569} and OD_{600} is obtained for a given subject from I_{in} and I_{out} values obtained for first and second images 13 and 17 using the following equation:

$$OD_{vessel} = \log_{10} (I_{out} / I_{in})$$

where OD_{vessel} is the optical density of a vessel, I_{out} is the average intensity outside the vessel, and I_{in} is the average intensity inside the vessel. The values of ODR_{vessel} are then determined by direct calculation from the data collected as follows:

$$ODR_{vessel} = OD_{vessel,600} / OD_{vessel,569}.$$

Because ODR and O_2SAT have a linear relationship, a linear regression analysis may be performed on ODR_{vessel} values at various systemic O_2SAT levels (obtainable from a common pulse oximeter) to yield the slope of the linear regression line of ODR vs. O_2SAT, which constitutes an estimate of OS (oxygen sensitivity). Such a model gives excellent agreement with the change in ODR_{vessel} with respect to systemic O_2SAT between 77% and 100%, falling along straight lines with a goodness of fit ("R") of close to minus one. As shown in FIG. 13, regression lines from different subjects each resulted in excellent agreement with a particular

linear relationship between ODR and systemic O_2SAT, causing slopes and intercepts of each line to differ. The generation of the data represented in FIG. 13 is discussed in the example presented below. We found that standard errors of ODR_{vessel} values under this analysis are typically less than 3% of the mean when results from four or more images are averaged.

With the OS value known for a particular vessel of a particular subject, O_2SAT_{vessel} may be calculated using the slope, OS, and intercept, Int, of the regression line as follows:

$$O_2SAT_{vessel} = (ODR_{vessel} - Int) / OS$$

Regression coefficients will need to be obtained for each new vessel by a separate calibration. Hence, although the linear response of the oximeter has been established with the direct reflectance method, it may be impractical to use this method to study a large number of subjects, as is needed in clinical evaluations and research investigations. To overcome this limitation of the direct reflectance method, the present invention contemplates that the amount of pigment contained in extravascular structures will vary from subject to subject, and will have an effect on measured OD_{vessel} values which influence the relationship between ODR_{vessel} and O_2SAT_{vessel}. It is noted that presence of extravascular pigment causes underestimation of the vessel OD at 569 nm, a wavelength at which pigment light absorption is strong. In addition, variability in the relationship between ODR_{vessel} and O_2SAT_{vessel} among the population masks the effects which vessel size has upon the relationship between ODR_{vessel} and O_2SAT_{vessel} in paired arteries and veins from which data is extracted. This effect of vessel size has a significant influence upon accuracy of the O_2SAT_{vessel} measurement where the vessel diameters differ by a large factor.

Pigmentation Correction Method

The pigmentation correction method of the present invention overcomes the

disadvantages of the direct reflectance method. The pigmentation correction method significantly reduces influence of pigmentation upon the relationship between ODR_{vessel} and O_2SAT_{vessel}. The pigmentation correction method of the present invention provides such a correction, as well as a correction for the vessel diameter.

The pigmentation correction method of the present invention assumes that extravascular light reflectance at the first wavelength (e.g., 600 nm) is influenced to a significantly lesser degree by pigment light absorption than is the reflectance at the second wavelength (e.g., 569nm), where pigment light absorption is strong. To calibrate the system using the pigmentation correction method, a corrected value for OD_{vessel} at 569 nm ("$OD_{cor, 569}$") is calculated using the following formula for each artery and vein pair in each subject:

$$OD_{cor, 569} = \log_{10} (\eta I_{out, 600} / I_{in, 569})$$

where η is the ratio of transmission from the light source to each of the images inherent in the image splitter and camera. Thus, an estimate of reflectance at 569 nm is obtained from the 600 nm reflectance. The value of η therefore need only be calculated once for a given image splitter and camera system, and may be determined by measuring intensities of the two images reflecting off a neutral reflective surface (e.g., $BaSO_4$), as follows:

$$\eta = (I_{in,600} - I_{dark}) / (I_{in,569} - I_{dark})$$

where I_{dark} is the average intensity within the area L of the scanned fundus image (i.e. the dark line denoted as "L" in FIG. 10). A vessel OD ratio corrected for pigmentation effects ("ODR_{cor}") is then obtained as follows:

$$ODR_{cor} = OD_{600} / OD_{cor, 569}.$$

The ODR_{cor} values are plotted against systemic O_2SAT, values of which are known from pulse oximeter measurements as described previously. A linear regression analysis may then be performed between these variables, and slopes of regression may be found in the conventional manner. In practice, it has been found that when values of ODR_{cor} are

determined with respect to several subjects using this method and are plotted against systemic O_2SAT, the variance of linear regression coefficients between subjects is significantly reduced with respect to those obtained using uncorrected ODR values (e.g. using the direct reflectance method).

Therefore, it has been found that the mean value of regression slopes can serve to represent the oxygen sensitivity, OS, which is inherent in the oximeter response. Plots of regression lines from several subjects, shown in Fig. 14, document the degree of agreement between regression slopes obtained in a group of subjects having skin pigmentation varying from fair to dark, and iris pigmentation of blue and brown. The generation of the data represented in FIG. 14 is discussed in the example presented below. The degree of improvement is noticeable by comparing this plot with that of the direct reflectance method shown in FIG. 13. Thus, removal of the influence of extravascular pigmentation, which is present when extravascular light reflectance at 569 nm is included, results in a consistent OS value for measurements from the general population. It is also apparent that the pigmentation correction method reduces, but does not eliminate, the range of vertical offsets between the regression lines. The invention anticipates that by measurement of the vessel ODR_{cor} during breathing of 100% oxygen, the correct amount of vertical offset can be ascertained. By this method then, the vertical offset is first determined during oxygen breathing, and the value of O_2SAT is then determined under desired conditions by a second measurement. Then OS, which is determined from the mean of regression slopes determined earlier from calibration measurements, is applied to make the particular desired measurement of O_2SAT in the desired vessel.

Once the mean best fit of the slopes, OS, is found, the value of O_2SAT_{vessel} is found by the following equation:

$$O_2SAT_{vessel} = 100\% - (ODR_{cor,\ artery} - ODR_{cor,\ vessel} - (\Delta D \times C)) / OS$$

where ΔD is the difference in diameters of the vessels and C is the slope of the line fit between $ODR_{cor, artery}$ at 100% oxygen and diameter as shown in FIG. 15 (upper panel), i.e., vessel diameter sensitivity. This equation takes into account the vertical offsets in measurements of ODR_{cor} at 100% O_2SAT by adjusting the ODR_{vessel} value according to the relationship between ODR at 100% O_2SAT and vessel diameter ("D"). The equation therefore corrects the measurement for any effects caused by differences in vessel diameter.

The vessel diameter, D, is found from the image by determining horizontal and vertical distances ("D_h" and "D_V", respectively) between points where light intensity is midway between values inside and outside vessel, and applying the formula $1 / D^2 = 1 / D_h^2 + 1 / D_V^2$. The value of ΔD is difference in the value of D for two vessels, in particular for one artery for which measurement was made with 100% breathing oxygen, and for a second vessel for which measurement is made under desired conditions. Correction for D is thus based on measurement of the vessel diameters and upon the linear regression fit between the ODR_{cor} at 100% O_2SAT and vessel diameter, and this correction is included in the equation as a factor which adjusts the value of the ODR_{cor} by an amount predicted by the relationship established through the regression fit between the value of ODR_{cor} measured in the artery at 100% and vessel diameter. This method of pigmentation correction provides means to utilize the oximeter for measurement of O_2SAT in arteries and veins of the general population under desired measurement conditions, which include those presented by experimental means and those presented by disease conditions.

Intravascular Reflectance Method

Another method for calibrating the system to avoid the effects of pigmentation and vessel diameter is the intravascular reflectance method. This approach involves comparing reflectance from only within blood vessels at the two chosen wavelengths, and replacing measured reflectance outside vessels with an estimate of reflectance from

unpigmented fundus.

Assuming that arterial OD at 100% O_2SAT_{artery} is primarily dependant upon light absorption by oxyhemoglobin, an appropriate estimate of unpigmented reflectance ("UR") would set vessel ODs at each wavelength to values predicted from the oxyhemoglobin absorption spectrum. Using the following equation, values of UR may be evaluated from hemoglobin light extinction coefficients which are determined for the filter characteristics of the oximeter, and from measured reflectance values from inside vessels at each wavelength when blood is 100% saturated with oxygen:

$$\varepsilon_{HbO2,\,600} \;/\; \varepsilon_{HbO2,\,569} = ODR_{irm,100\%}$$

$$= \log (UR / I_{in,\,600,\,100\%}) \;/\; \log (UR / I_{in,\,569,\,100\%})$$

$$= 0.0792$$

where $ODR_{irm,100\%}$ is an ODR value obtained using the intravascular reflectance method, UR is unpigmented reflectance, $I_{in,\,600,\,100\%}$ and $I_{in,5\,69,\,100\%}$ are average intensities obtained inside blood vessels in the 600 nm and 569 nm wavelength images, respectively, and obtained from images recorded during 100% oxygen breathing, and $\varepsilon_{HbO2,\,600}$ and $\varepsilon_{HbO2,\,569}$ are extinction coefficients of blood which are measured using first and second bandpass filters employed for wavelength selection at 600 and 569 nm.

The broadband extinction coefficients $\varepsilon_{HbO2,\,600}$ and $\varepsilon_{HbO2,\,569}$ are calculated from products of hemoglobin absorption spectra with the filter spectra as is known in the art and referenced in Delori, "Noninvasive Technique For Oximetry Of Blood In Retinal Vessels," Appl. Optics 27 (1988) at 1061-77. For the oxyhemoglobin spectrum, a polynomial regression is used to interpolate between light extinction points to produce a curve. Values from the curve are multiplied by filter response curves to obtain the coefficients. These values were obtained for the imaging ocular oximeter of the embodiment of FIGS. 1 - 9, and were compared with the coefficients of Van Assendelft obtained at high

spectral resolution (see Van Assendelft, "Spectrophotometry of Hemoglobin Derivatives," (C.C. Thomas 1970)), and are shown in the following table along with the narrow band values for purposes of comparison:

TABLE I

	Wide Band		Narrow Band	
	569 nm	**600 nm**	**569 nm**	**600 nm**
ε_{Hb}	10.75	3.0	11.72	3.2
ε_{HbO2}	9.85	0.78	11.72	0.8

Initially, the equation set forth above is used with measurements of I_{in} obtained from images recorded during 100% oxygen breathing to calculate UR for each subject. Once the UR values have been obtained, the above equation is again employed for a given subject, using the UR value obtained for that subject, to obtain values of ODR_{irm} for systemic O_2SAT_{artery} values other than 100% O_2SAT_{artery}. This is done for each subject, and a plot of ODR_{irm} vs. O_2SAT_{artery} is obtained for each. A line of best fit is determined for each of these plots. As with the pigmentation correction method, a value of oximeter response OS is then determined from the simple mean of best fit line slopes. In this case, however, correction for vessel diameter is obtained by adjusting the value of the slope according to the relationship between regression slopes and diameter.

The other difference between the pigmentation correction method and this method is that the second measurement using 100% oxygen breathing is not necessary. This step is instead included in the model assumption and, as can be seen from the regression lines obtained by this method in FIG. 16, all the lines converge to the value of the ODR predicted by the model at 100% O_2SAT_{artery}. Thus, when regression lines are plotted together, the effect of vessel diameter upon the slope of the lines is readily apparent (FIG. 16). As will be described in the Example set forth below, a statistically significant correlation between these

slopes and the vessel diameter, D, was determined in a study from vessels of differing size in a group of calibration subjects having wide variations in pigmentation. The new regression line through the points shown in Fig. 15 (lower panel) represent the sensitivity of the individual slopes of regressions obtained from the calibration subjects with respect to vessel diameter. The value of the slope of this new regression line, the vessel diameter sensitivity, is noted as C in the discussion below.

The process of using the intravascular reflectance method involves first establishing the mean value for OS and the value for C. The value of O_2SAT in a particular vessel under desired measurement conditions may then be found by first measuring the ODR_{irm} for the vessel and locating this value on the y axis of a plot with respect to systemic O_2SAT. The diameter of the vessel is measured as described above for the pigmentation correction method. This value of D is used to correct the mean value of the slope obtained during calibration measurements in different subjects. This correction is obtained by the following equation:

$$OS_{cor} = OS + \Delta D \times C,$$

where C is the slope of the regression line as defined above. The intravascular reflectance method thus also enables measurement of O_2SAT_{vessel} from the general population once initial calibrations with the oximeter are performed. This method has advantages of requiring only reflectance readings from within the blood vessels, which simplified the process of scanning the image. Exclusion of measurements from extravascular areas also reduces spurious influence upon the measurement from specular reflections outside vessels, such as from nerve fibers near the surface of the tissue.

As indicated previously, persons having skill in this art will readily appreciate that, in practice, the empirical value OS derived from the pigmentation correction method or intravascular reflectance method need only be obtained once using a statistically significant population and appropriate scientific methods to ensure accuracy. This value, along with the

forgoing formula for calculating O_2SAT_{vein} or ΔO_2SAT_{vein}, may then be embedded in a computer program for generating a result for research or diagnostic purposes evaluating the oxygen utilization (e.g., oxygen supply, delivery, consumption, and gradients of O_2SAT along vasculature) in the tissue of the retina or other portions of the posterior pole of the eye.

Accordingly, in practice, a device of the type described above is used to perform the image acquisition step of the present invention, obtaining simultaneous first and second images 13 and 17 of a patient's retinal blood vessels using imaging device 16, which preferably is a digital camera. A search algorithm embedded in the computer program may then be used to perform the scanning step of the invention, in which first and second images 13 and 17 are scanned and I_{in} and I_{out} values obtained. If the requisite OS value has not yet been obtained, the computer program may then be further used to calculate ODR values using the direct reflectance, pigment correction or intravascular reflectance methods, or another calibration method which preferably corrects for pigmentation and/or vessel size. Where the requisite OS value has been obtained, the computer program may also be programmed to calculate a value for O_2SAT_{vein} using data collected for a subject. In a clinical setting, this value may be compared to available data concerning diseases affecting the posterior pole of the eye, and a diagnosis made.

From the foregoing, it will be apparent that the present approach differs from those previously employed in several important respects, including the use of an image splitter and digital image method of reflectance at two carefully wavelengths to determine empirical relationships between ODR and O_2SAT values, avoiding assumptions about the optical path through a vessel. The system is compact, low-cost, and simple to use. In addition, the present approach uniquely features the ability to compensate for the variability in fundus pigmentation among different individuals, as well as for vessel diameter, using a device and method that are simpler in theory and practice and require less complex

instrumentation than those previously described. This approach yields reasonable estimates of O_2SAT in both the larger retinal arteries and veins, and should be applicable to vessel sizes of between about 50μm and 200μm. The present invention also makes possible understanding the effects of metabolism or blood flow changes on retinal function by enabling the determination of changes in vessels occurring before and after interventions, a change which is made straightforward to obtain since many variables potentially affecting absolute measures of O_2SAT will cancel. Although the values derived from the empirical relationships disclosed and utilized in the present invention will vary somewhat depending upon the size and composition of the pool of subjects from which data is collected, an adequate sample size and inclusion of light and dark pigmented subjects will ensure the validity of these empirical relationships. The following provides an example of the calculation of such values.

EXAMPLE

Oximetry data was collected in a study of ten volunteers using the imaging ocular oximeter illustrated in FIGS. 1-9. The volunteers were healthy, non-smoking males aged 18-40 years and having no history of dyshemoglobinemia. One subject was unable to complete the oximetry portion of the study. Two subjects were excluded from the oximetry portion of the study because images obtained from one of these subjects revealed sclerosis of the arterial wall, and in the other subject the artery demonstrated extreme instability of caliber during measurements. Oximetry data from five Caucasian subjects, three of northern European descent and two of southern European descent, and two African-American subjects were included in the study. ARVO guidelines for human investigation were followed, and the study protocol was approved by the Human Investigation Committee of the University of Virginia.

For each of the subjects, an Ohmeda 3700 (version J) ear oximeter probe was

placed on the massaged earlobe. Although a finger probe could have been used instead, an ear probe was chosen because of its ability to respond accurately and quickly to a changing systemic O_2SAT_{artery}. The Ohmeda 3700 in particular features a graphic display of waveform and signal strength to aid in minimizing inaccurate readings. As illustrated schematically in FIG. 12, a Mapleson C breathing circuit 134 and face mask 136 was modified by inserting an inspiratory valve 138 just proximal to the mask, allowing all exhaled gas to be vented through an expiratory valve 140 placed just distal to face mask 136. This arrangement of components kept rebreathing of alveolar gas and dead space to a minimum. A polarographic inspired oxygen analyzer 142 with a Clark electrode (Critikon Oxychek model 2000) was calibrated to room air and inserted just proximal to inspiratory valve 138. Velcro straps (not shown) were used to fix face mask 136 tightly over a subject's mouth and nose. A fresh gas flow port 144 was positioned between oxygen analyzer 142 and a reservoir bag 146. Reservoir bag 146 was inflated with the gas mixture before breathing started.

Subjects were allowed to breathe room air during the first series of fundus image recordings. Various oxygen/nitrogen mixtures (size H gas cylinders) were delivered to the subjects using flows of at least about 10 liters/minute. Following the initial image recordings breathing room air, subjects breathed either a gas mixture containing 14% oxygen, which corresponds to an arterial hemoglobin O_2SAT of approximately 94%, or a mixture of 10% oxygen, which corresponds to a hemoglobin O_2SAT of approximately 84%. In some individuals, the 14% oxygen mixture was given followed by the 10% oxygen mixture. A mixture containing 8% oxygen was then given, which corresponds to a hemoglobin O_2SAT of approximately 80%. Finally, subjects breathed 100% oxygen, which produces an arterial hemoglobin O_2SAT of 100%. When a steady state was achieved at each step (defined by an unchanging pulse oximeter reading for 2 minutes), the pulse oximeter systemic O_2SAT reading was noted.

The eyes of each subject were dilated with topical tropicamide 1% and phenylephrine 2.5%. After the pupil diameter stabilized, the room was darkened and the subject's fundus was illuminated using the tungsten aiming light (not shown) of fundus camera 10. The eye gaze was positioned to enable imaging the retinal area of interest using a movable illuminated fixation target against a dark background. Optimal focus was also achieved using the aiming light. The actual image recording at each breathing gas mixture was made using xenon flash 30. Three to five dual wavelength images (comprising first and second images 13 and 17) were recorded at each gas mixture and stored to the disc memory of a computer. Each image element formed by imaging device 16 was formed with 3x3 pixels, with the camera temperature maintained at -60°C. The scanning step of the invention was performed as described previously using the computer program set forth in full in Appendix A hereto.

Values of I and OD_{600} and OD_{569} were also calculated using this program. Using the direct reflectance method described herein, the ratio OD_{600} / OD_{569} was calculated by the program and plotted against values of systemic O_2SAT that had been measured with the pulse oximeter, as can be seen in FIG. 13. The slopes of the regression lines ranged between 0.00427 to 0.01242 (μ = 1.02, SE = 0.00098) and y-intercepts (ODR at O_2SAT = 0%) ranged between 0.514 - 1.50 (μ = 1.02, SE = 0.115). The R value (goodness of fit) ranged between -0.9581 and -.9999 with an average of -0.98318, as shown in Table II below, in which "p" is a confidence limit.

TABLE II

Diameter (μm)	Slope (x10^5)	Y-Intercept (0% 0_2)	ODR (100% 0_2)	R	P
185	982	1.32	0.34	-0.9927	0.0073
185	427	0.60	0.17	-0.9999	0.0001
150	727	1.00	0.28	-0.9674	0.0016
139	661	0.93	0.20	-0.9762	0.0237
127	1242	1.50	0.27	-0.9955	0.0045
105	672	0.81	0.14	-0.9581	0.0419
100	801	0.99	0.19	-0.9924	0.0076

Thus, the linear model was in excellent agreement with the change in ODR vs. systemic O_2SAT between 77% and 100% saturation. Maximum and minimum slopes varied by a factor of 2.91 and the coefficient of variation in regression slope (SE/μ) was equal to 0.125. Standard errors of IDRs were typically less than 3% of the mean when results from four or more images were averaged. Using these empirical values, O_2SAT values were calculated as described above. The use of the optical density ratio also avoids adverse effects of eye motion, which cause vessels to become misfocused. This adverse effect is overcome because any misfocus is present in both images, and influence of misfocus is therefore canceled in the ratio.

A pigmentation correction method, as described previously, was used to correct for pigmentation among the various subjects, as well as for vessel diameter. For calculating $ODR_{cor,\,569}$, η was calculated to be 1.79 for the embodiment of the imaging retinal oximeter system described in FIGS. 1 through 9. When values of ODR_{cor} from all subjects were calculated by the computer program and plotted against O_2SAT, the variance of linear regression coefficients between subjects was significantly reduced with respect to those obtained using the uncorrected ODR. Slopes ranged between 0.00433 and 0.00633 (μ =

0.00504, SE = 0.00029, coefficient of variation = 0.057) and y-intercepts between 0.492 and 0.775 (μ = 0.617, SE = 0.033). The correction did not significantly change the standard errors of points for individuals. Figure 14 shows ODR_{cor} points from the same seven subjects (each type of symbol represents a different subject) with line fits (dotted lines), along with the oxygen sensitivity OS (solid line "OS") averaged from individual slopes and intercepts. Results of a regression analysis are shown in Table III below:

TABLE III

Vessel Diameter (μm)	Slope ($x10^5$)	Y-Intercept (0% 0_2)	ODR (100% 0_2)	R	P
185	633	0.78	0.14	-0.9971	0.0029
185	459	0.59	0.13	-0.9999	0.0108
150	446	0.61	0.17	-0.9444	0.0046
139	486	0.61	0.13	-0.9760	0.0240
127	586	0.67	0.86	-0.9730	0.0270
105	433	0.49	0.06	-0.9834	0.0166
100	488	0.58	0.09	-0.9947	0.0005

These results indicate that light absorption from retinal pigments, which is especially strong near 569 nm, influences determinations of O_2SAT_{vessel} from reflectance measurements, and show that a correction for pigment variation, based on estimation of incident illumination at both wavelengths using values measured at 600 nm, provides a two-fold reduction in the variability of oxygen sensitivity among subjects obtained by the present method over that obtained using the direct reflectance approach.

To determine if the range in oxygen sensitivity remaining after pigment correction could have resulted from different vessel sizes, a second linear regression of line fit parameters against vessel diameter was performed, as shown in FIG. 15 (upper panel).

Significance ($p < 0.05$) was obtained for values of ODR_{cor} obtained breathing 100% oxygen. There was a positive trend between the slope of the regression and diameter which was not statistically significant ($p = 0.21$). These results support the conclusion that the range in ODR_{cor} values which was associated with a given value of systemic O_2SAT was partially the result of different vessel sizes, and that points in this range can be correlated to a measurement obtained under repeatable conditions (e.g., 100% breathing oxygen).

Values of O_2SAT_{vein}, corrected for pigmentation and vessel diameter were determined using the values of C and OS empirically found using the pigmentation correction method. The value of C was 0.0129 / pixel, and OS was 0.00504 / unit of O_2SAT (%). Results of this analysis are summarized in Table IV below for room air breathing by each subject:

TABLE IV

Arterial O_2SAT (room air) (%)	Vessel Diameter (Artery) (μm)	Vessel Diameter (Vein) (μm)	ODR_{cor} (Artery)	ODR_{cor} (Vein)	Arteriovenous O_2SAT Difference (%)	Venous O_2SAT (room air) (%)
97	185	209	0.143	0.439	54	43
98	139	139	0.119	0.339	44	54
95	100	131	0.860	0.290	34	61
97	105	150	0.560	0.250	35	62
99	150	175	0.165	0.401	44	55

Further improvement in the accuracy of this approach may be obtained by incorporating the vessel size dependence of oxygen sensitivity. However, the present correlation between the regression slope of ODR_{cor} and vessel diameter is not statistically significant, and the mean value of slopes may therefore be used to define the oxygen sensitivity, and only the dependence of ODR at 100% O_2 with respect to vessel diameter may be used to correct for different vessel sizes.

The results of Table IV show a range in O_2SAT_{vein} of approximately 26% among subjects with a mean value of 57%. The increase in O_2SAT_{vein} caused by breathing 100% O_2 (hyperoxia relative to normoxia) was determined by subtracting the normoxic value of ODR_{cor} from the hyperoxic value, and dividing by OS. In this case, size-dependent offsets in the O_2SAT_{vein} were canceled by subtraction and no correction for vessel size was needed. As shown in Table V below, hyperoxia (100% oxygen breathing) was found to increase venous O_2SAT by 19.2 ± 2.9 %. The smallest increase occurred in the subject with the highest saturation at rest.

TABLE V

Arterial O_2SAT (room air) (%)	**Venous ODR_{cor}**		**Venous O_2SAT Difference (100% O_2 - room air) (%)**	**Venous O_2SAT (100% O_2) (%)**
	(100% O_2)	**(room air)**		
99	0.348	0.439	19	62
98	0.225	0.339	23	77
95	0.175	0.290	23	84
97	0.211	0.250	8	70
99	0.283	0.401	23	78

An intravascular reflectance method was also independently employed to calculate results unaffected by extravascular pigmentation. Measurements of I_{in} were obtained at 100% oxygen breathing to calculate UR by the iterative method of this method. Values for ODR_{irm} for the paired veins and arteries of the subjects were then obtained. FIG. 16 shows the regression lines which were fit to the data points for each subject, along with the diameters of each artery (A = 105 μm; B = 100 μm; C = 139 μm; D = 150 μm; E = 127 μm; F = 185 μm; G = 185 μm). Initial assumptions result in the lines meeting near 100% systemic O_2SAT, and thus show more clearly the range in slopes from the subjects. Regression intercepts and slopes can be seen to be co-dependent. The results are shown in

Table VI below:

TABLE VI

Diameter (μm)	Slope (x10^5)	Y-Intercept (0% 0_2)
185	616	0.695
185	560	0.616
150	480	0.565
139	461	0.554
127	512	0.594
105	437	0.513
100	481	0.559

Slopes had a mean value $\mu = 0.00506$, a standard error SE = 0.00023, and a coefficient of variation = 0.045. These results again suggest that there is a small dependence of the oxygen sensitivity upon size of vessels. With the OS value determined in this manner, values of O_2SAT_{vein} could be calculated as described in connection with the pigmentation correction method.

Among other potential uses, the device and method of the present invention have applicability as tools for medical research, e.g., in studies of oxygen utilization in the human retina, and as a diagnostic tool for humans or animals with respect to many eye diseases. Its potential clinical applications are particularly important, as a uniquely noninvasive system for evaluating oxygen metabolism in human patients during eye examinations and screening for the risk of eye diseases. The invention will be particularly applicable for clinical tests for assessing the risk for onset of diabetic retinopathy, and for tests evaluating the effects of topical medications on blood flow and oxygen delivery in the retina. The present ocular vessel oximeter and method will be thus useful in any research or

clinical application where hemoglobin O_2SAT must be determined in the larger (approximately 50 μm to 200 μm) range of retinal blood vessels.

APPENDIX A

Source Code From "Software For An Imaging Ocular Vessel Oximeter"
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```
Program retinal_vessel_od_measurement;
uses dos, crt;
   {$M 53400, 0, 655360}
type
  ves_array = array [1..341] of word;
var
  avg3,avg4,evavg6,evavg5,minsum5,minsum6:ves_array;
  od569,od600,r,pr,pod569:real;
  di,vw,offset: integer;
  scanlength,dx,dy,x569,y569,x600,y600,vi600,vi569,evi569,evi600:longint;
  infile, outfile : file;
  datafile,output : string[30];
  result:text;
  p,direction:char;
  vein:boolean;

procedure getinput(var x600,x569,y600,y569,dx,dy,scanlength:longint; var
p,direction:char;
var vw,offset:integer;var vein:boolean);
var
 a:char;
 begin{getinput}
   vein:=true;
   writeln('Enter the image file');
   readln(datafile);
   repeat
     writeln('Enter (a) for artery and (v) for vein');
     readln(a);
   until (a= 'a') or (a='v');
   if a='a' then
     vein:=not(vein);  {then artery}
   repeat
     writeln('Enter (h) for horizontal and (v) for vertical orientation');
     readln(direction);
   until (direction='h') or (direction ='v');
   writeln('Enter scanlength, vessel width, dark current');
   readln(scanlength,vw,di);
   writeln('Enter starting x and y coordinates of the vessel at 600 nm');
   readln(x600,y600);
   writeln('Enter starting x and y coordinates of the vessel at 569 nm');
   readln(x569,y569);
   if direction = 'v' then
```

```
        begin
         dx:=x569-x600;
         dy:=0;
        end;
      if direction = 'h' then
        begin
         dy:=y569-y600;
         dx:=0;
        end;
      if direction ='v' then
        begin
         repeat
          writeln('Sample left(l), right(r), or both(b) sides of vessel to estimate EV?');
          readln(p);
         until (p='l') or (p='r') or (p='b');
        end;
      if direction ='h' then
        begin
         repeat
          writeln('Sample top(t), bottom(b), or both(B) sides of vessel to estimate EV');
          readln(p);
         until (p='t') or (p='b') or (p='B');
        end;
      writeln('Enter offset length from vessel to sample EV');
      readln(offset);
    end;{getinput}

procedure init_textfiles(var result:text;var
infile,outfile:file;direction:char;vein:boolean);
 var
output : string[30];
test:text;

begin{init_textfiles}
    assign(infile,datafile);
    assign(test,'test.txt');
    rewrite(test);
    if vein=false then
      begin
       if direction='v' then
        begin
         writeln(test,datafile,'va.txt');
         reset(test);
         readln(test,output);
        end;
```

```
      if direction = 'h' then
       begin
        writeln(test,datafile,'ha.txt');
        reset(test);
        readln(test,output);
       end;
     end;
    if vein = true then
     begin
      if direction='v' then
       begin
        writeln(test,datafile,'vv.txt');
        reset(test);
        readln(test,output);
       end;
      if direction = 'h' then
       begin
        writeln(test,datafile,'hv.txt');
        reset(test);
        readln(test,output);
       end;
     end;
    assign(result,output);
    rewrite(result);
    if vein=false then
     begin
      if direction = 'v' then
       writeln(result,'    Artery Analysis(Vertical)');
      if direction = 'h' then
       writeln(result,'    Artery Analysis(Horizontal)');
     end;
    if vein=true then
     begin
      if direction = 'v' then
       writeln(result,'    Vein Analysis(Vertical)');
      if direction = 'h' then
       writeln(result,'    Vein Analysis(Horizontal)');
     end;
    writeln(result);
    writeln(result,'Data record of file ',datafile);
    writeln(result,'Scanlength = ',scanlength,', vessel width = ',vw);
    writeln(result,'Dark current = ',di);
    writeln(result,'Starting coordinates at 600 nm (x,y) = ',x600,',',y600);
    writeln(result,'Starting coordinates at 569 nm (x,y) = ',x569,',',y569);
    if direction ='v' then
```

```
      begin
       if p='l' then
          writeln(result,'Left side of vessel was measured to estimate evi at offset =
',offset);
          if p='r' then
          writeln(result,'Right side of vessel was measured to estimate evi at offset =
',offset);
          if p='b' then
        writeln(result,'Both sides of vessel were measured to estimate evi at offset =
',offset);
      end;
    if direction ='h' then
      begin
       if p='t' then
       writeln(result,'Top side of vessel was measured to estimate evi at offset =
',offset);
       if p='B' then
       writeln(result,'Both sides of vessel was measured to estimate evi at offset =
',offset);
       if p='b' then
       writeln(result,'Bottom side of vessel were measured to estimate evi at offset =
',offset);
      end;
    reset(infile,1);
    close(test);

end;{init_textfiles}

procedure scanartery_vertical(var infile:file;var
x600,x569,y600,y569,dx,scanlength:longint;p:char;
vw,offset:integer;var avg3,avg4,evavg5,evavg6,minsum5,minsum6:ves_array;var
result:text);

var
 dx5,j,l,i,min569,min600,s,t:integer;
 min,evs5,evs6:word;
 imagepos,evlsum5,evlsum6,evrsum5,evrsum6,evsum5,evsum6,sum3,sum4,
 minv5,maxv5:longint;
 read600,read569,avg569,avg600,temp5,temp6,ev5,ev6: array[1..341] of word;
 sample569,sample600,ev600,ev569: array[1..3,1..3] of word;
 next569,next600: array[1..3] of word;
 out9,out10:text;

procedure detect_arterialwall_vertical(x569,y569:longint;vw:integer;var
minv5,maxv5:longint);
```

```
var
  sobely,sobelx: array[1..3,1..3] of integer;
  imagepos,evlsum5,evlsum6,evrsum5,evrsum6,evsum5,evsum6,sum3,sum4:longint;
  read569:array[1..3,1..341] of integer;
  detect5:array[1..341] of integer;
  a,i,j,k,gx5,gy5,max569,min569,min5,max5:integer;
  wall:boolean;


begin{detect_arterialwall_vertical}
   sobely[1,1]:=-1; sobely[1,2]:=0; sobely[1,3]:=1;
   sobely[2,1]:=-2; sobely[2,2]:=0; sobely[2,3]:=2;
   sobely[3,1]:=-1; sobely[3,2]:=0; sobely[3,3]:=1;
   sobelx[1,1]:=-1; sobelx[1,2]:=-2; sobelx[1,3]:=-1;
   sobelx[2,1]:=0; sobelx[2,2]:=0; sobelx[2,3]:=0;
   sobelx[3,1]:=1; sobelx[3,2]:=2; sobelx[3,3]:=1;

   for i:=1 to 3 do
    begin
     for k:= 1 to vw*2+1 do
      begin
       imagepos:= 512+ 4*(x569-vw-1+k) + 341*(y569-2+i)*4  ;
       seek(infile,imagepos);
       blockread(infile,read569[i,k],4);
      end;
     min569:=x569+vw;
     max569:=x569-vw;
    end;
   for k:= 2 to vw*2 do
    begin

gy5:=(read569[1,k-1]*sobely[1,1]+read569[1,k]*sobely[1,2]+read569[1,k+1]*sobely[
1,3]+read569[2,k-1]*sobely[2,1]+read569[2,k]*sobely[2,2]+read569[2,k+1]*sobely[2
,3]
+read569[3,k-1]*sobely[3,1]+read569[3,k]*sobely[3,2]+read569[3,k+1]*sobely[3,3])
;

gx5:=(read569[1,k-1]*sobelx[1,1]+read569[1,k]*sobelx[1,2]+read569[1,k+1]*sobelx
[1,3]+read569[2,k-1]*sobelx[2,1]+read569[2,k]*sobelx[2,2]+read569[2,k+1]*sobelx[
2,3]
+read569[3,k-1]*sobelx[3,1]+read569[3,k]*sobelx[3,2]+read569[3,k+1]*sobelx[3,3])
;

      detect5[k]:=(gx5+gy5) div 9;
        end;
```

```
min5:=maxint;
max5:=-maxint;
for k:=3 to vw+3 do
 begin
      if detect5[k] < min5 then
   begin
    min569:=x569-vw-1+k;
    min5:=detect5[k];
   end;
    end;
wall:=false;
for k:=vw*2-2 downto vw-2 do
 begin
  if (detect5[k] > max5) and (wall = false) then
   begin
        max5:=detect5[k];
        max569:=x569-vw-1+k;
    if abs(vw-max569+min569) <= 1 then
     begin
      max5:=-maxint;

      if ((vw-max569+min569=-1) and (detect5[k] >= detect5[k-1]) and
      (detect5[k] >= detect5[k-2])) or ((vw-max569+min569=0) and (detect5[k]
>= detect5[k-1]))
      or (vw-max569+min569=1) then

            wall:=true;
     end;
   end;
 end;
minv5:=min569;
maxv5:=max569;

end;{detect_arterialwall_vertical}

begin {scanartery}
   assign(out9,'valine6.dat');
   rewrite(out9);
   assign(out10,'valine5.dat');
   rewrite(out10);
   if p= 'b' then
    begin
     writeln(out10,'vessel min(x) vessel   y');
     writeln(out9, 'vessel min(x) vessel   y');
    end;
```

```
if p='r' then
 begin
  writeln(out10,'vessel min(x) vessel  y');
  writeln(out9, 'vessel min(x) vessel  y');
 end;
if p='l' then
 begin
  writeln(out10,'vessel min(x) vessel   y');
  writeln(out9, 'vessel min(x) vessel   y');
 end;
l:=0;
repeat
l:=l+1;
detect_arterialwall_vertical(x569,y569,vw,minv5,maxv5);
dx5:=x569-minv5;
for j:=1 to maxv5-minv5-1+4 do
 begin
  imagepos := 512+ 4*(minv5+j-2) + 341*y569*4 ;
      seek(infile,imagepos);
      blockread(infile,read569[j],4);
 end;
min569:=dx5-1;
for i:=dx5-1 to dx5+4 do
 begin
  if read569[min569]>read569[i+1] then
  min569:=i+1;
 end;
x569:=x569+min569-2-dx5;
x600:=x569-dx;
for i:= 1 to 9 do
 begin
  imagepos := 512+ 4*(x600-5+i) + 341*y600*4 ;
  seek(infile,imagepos);
  blockread(infile,read600[i],4);
 end;
min600:=5;
for i:=3 to 7  do
     if read600[min600]>read600[i] then
  min600:=i;
x600:=x600-5+min600;
avg569[l]:=read569[min569];
avg600[l]:=read600[min600];
sum4:=0;
sum3:=0;
evlsum5:=0;
```

```
evlsum6:=0;
evrsum5:=0;
evrsum6:=0;
for s:=1 to 3 do
    for t:= 1 to 3 do
  begin
      imagepos := 512+ 4*(x569-2+s) + 341*(y569-2+t)*4;
      seek(infile,imagepos);
      blockread(infile,sample569[s,t],4);
   imagepos := 512+ 4*(x600-2+s) + 341*(y600-2+t)*4;
   seek(infile,imagepos);
   blockread(infile,sample600[s,t],4);
      if p='l' then
    begin
     imagepos := 512+ 4*(x569-2+s-offset) + 341*(y569-2+t)*4;
         seek(infile,imagepos);
         blockread(infile,ev569[s,t],4);
     imagepos := 512+ 4*(x600-2+s-offset) + 341*(y600-2+t)*4;
     seek(infile,imagepos);
     blockread(infile,ev600[s,t],4);
         evlsum5:=evlsum5+ev569[s,t];
         evlsum6:=evlsum6+ev600[s,t];
             evsum5:=evlsum5;
             evsum6:=evlsum6;
       end;
      if p='r' then
    begin
     imagepos := 512+ 4*(x569-2+s+offset) + 341*(y569-2+t)*4;
         seek(infile,imagepos);
         blockread(infile,ev569[s,t],4);
     imagepos := 512+ 4*(x600-2+s+offset) + 341*(y600-2+t)*4;
     seek(infile,imagepos);
     blockread(infile,ev600[s,t],4);
         evrsum5:=evrsum5+ev569[s,t];
         evrsum6:=evrsum6+ev600[s,t];
             evsum5:=evrsum5;
             evsum6:=evrsum6;
       end;
   if p='b' then
    begin
     imagepos := 512+ 4*(x569-2+s-offset) + 341*(y569-2+t)*4;
         seek(infile,imagepos);
         blockread(infile,ev569[s,t],4);
     imagepos := 512+ 4*(x600-2+s-offset) + 341*(y600-2+t)*4;
     seek(infile,imagepos);
```

```
                blockread(infile,ev600[s,t],4);
                    evlsum5:=evlsum5+ev569[s,t];
                evlsum6:=evlsum6+ev600[s,t];
                imagepos := 512+ 4*(x569-2+s+offset) + 341*(y569-2+t)*4;
                seek(infile,imagepos);
                blockread(infile,ev569[s,t],4);
            imagepos := 512+ 4*(x600-2+s+offset) + 341*(y600-2+t)*4;
            seek(infile,imagepos);
            blockread(infile,ev600[s,t],4);
                evrsum5:=evrsum5+ev569[s,t];
                evrsum6:=evrsum6+ev600[s,t];
                    evsum5:=(evrsum5+evlsum5) div 2;
                    evsum6:=(evrsum6+evlsum6) div 2 ;
              end;
             sum4:=sum4+sample600[s,t];
             sum3:=sum3+sample569[s,t];
           end;
        temp5[l]:=sum3 div 9;
        temp6[l]:=sum4 div 9;
        ev5[l]:=evsum5 div 9;
        ev6[l]:=evsum6 div 9;
        if p='r' then
         begin
          writeln(out10,minv5:4,x569:8,maxv5:8,y569:8);
          writeln(out9,minv5-dx:4,x600:8,maxv5-dx:8,y600:8);
         end;
        if p='l' then
         begin
          writeln(out10,minv5:8,x569:8,maxv5:8,y569:8);
          writeln(out9,minv5-dx:8,x600:8,maxv5-dx:8,y600:8);
         end;
        if p='b' then
         begin
          writeln(out10,minv5:8,x569:8,maxv5:8,y569:8);
          writeln(out9,minv5-dx:8,x600:8,maxv5-dx:8,y600:8);
         end;
        y569:=y569+1;
        y600:=y600+1;
        until l=scanlength;
        for s:=1 to scanlength do
         begin
          avg3[s]:=temp5[s];
          avg4[s]:=temp6[s];
          evavg5[s]:=ev5[s];
          evavg6[s]:=ev6[s];
```

```
          minsum5[s]:=avg569[s];
          minsum6[s]:=avg600[s];
        end;
      close(out9);
      close(out10);
      close(infile);

end; {scanartery_vertical}

procedure scanartery_horizontal(var infile:file;var
x600,x569,y600,y569,dy,scanlength:longint;p:char;
vw,offset:integer;var avg3,avg4,evavg5,evavg6,minsum5,minsum6:ves_array;var
result:text);

var
 dy5,dy6,j,l,i,min569,min600,s,t:integer;
 temp:word;
 imagepos,evlsum6,evrsum5,evrsum6,evsum5,evsum6,sum3,sum4,evlsum5,
 minv5,maxv5,minv6,maxv6 :longint;
 read600,read569,avg569,avg600,temp5,temp6,ev5,ev6: array[1..341] of word;
 sample569,sample600,ev600,ev569: array[1..3,1..3] of word;
 next569,next600: array[1..3] of word;
 out9,out10:text;


procedure detect_arterialwall_horizontal(x569,y569:longint;vw:integer;var
minv5,maxv5:longint);
var
  sobely,sobelx: array[1..3,1..3] of integer;
  imagepos:longint;
  read569:array[1..3,1..341] of integer;
  detect5:array[1..341] of integer;
  i,j,k,gx5,gy5,max569,min569,min5,max5:integer;
  wall:boolean;

begin{detect_arterialwall_horizontal}
   sobely[1,1]:=-1; sobely[1,2]:=0; sobely[1,3]:=1;
   sobely[2,1]:=-2; sobely[2,2]:=0; sobely[2,3]:=2;
   sobely[3,1]:=-1; sobely[3,2]:=0; sobely[3,3]:=1;
   sobelx[1,1]:=-1; sobelx[1,2]:=-2; sobelx[1,3]:=-1;
   sobelx[2,1]:=0; sobelx[2,2]:=0; sobelx[2,3]:=0;
   sobelx[3,1]:=1; sobelx[3,2]:=2; sobelx[3,3]:=1;
   for i:=1 to 3 do
    begin
     for k:= 1 to vw*2+1 do
```

```
          begin
            imagepos:= 512+ 4*(x569-2+i) + 341*(y569-vw-1+k)*4  ;
            seek(infile,imagepos);
blockread(infile,read569[i,k],4);
          end;
        min569:=y569+vw;
        max569:=y569-vw;
       end;
      for k:= 2 to vw*2 do
       begin

gy5:=(read569[1,k-1]*sobely[1,1]+read569[1,k]*sobely[1,2]+read569[1,k+1]*sobely[
1,3]+read569[2,k-1]*sobely[2,1]+read569[2,k]*sobely[2,2]+read569[2,k+1]*sobely[2
,3]+read569[3,k-1]*sobely[3,1]+read569[3,k]*sobely[3,2]+read569[3,k+1]*sobely[3,
3]);


gx5:=(read569[1,k-1]*sobelx[1,1]+read569[1,k]*sobelx[1,2]+read569[1,k+1]*sobelx
[1,3]+read569[2,k-1]*sobelx[2,1]+read569[2,k]*sobelx[2,2]+read569[2,k+1]*sobelx[
2,3]+read569[3,k-1]*sobelx[3,1]+read569[3,k]*sobelx[3,2]+read569[3,k+1]*sobelx[
3,3]);

        detect5[k]:=(gx5+gy5) div 9;
          end;
      min5:=maxint;
      max5:=-maxint;
      for k:=3 to vw+3 do
       begin
            if detect5[k] < min5 then
         begin
          min569:=y569-vw-1+k;
          min5:=detect5[k];
         end;
          end;
      wall:=false;
      for k:=vw*2-2 downto vw-2 do
       begin
        if (detect5[k] > max5) and (wall = false) then
         begin
              max5:=detect5[k];
              max569:=y569-vw-1+k;
          if abs(vw-max569+min569) <= 1 then
           begin
            max5:=-maxint;
```

```
            if ((vw-max569+min569=-1) and (detect5[k] >= detect5[k-1]) and
            (detect5[k] >= detect5[k-2])) or ((vw-max569+min569=0) and (detect5[k]
>= detect5[k-1]))
            or (vw-max569+min569=1) then
                wall:=true;
          end;
        end;
      end;
    minv5:=min569;
    maxv5:=max569;

end;{detect_arterywall_horizontal}

begin {scanartery_horizontal}
    assign(out9,'haline6.dat');
    rewrite(out9);
    assign(out10,'haline5.dat');
    rewrite(out10);
    if p= 'B' then
      begin
        writeln(out10,'vessel min(y) vessel  x');
        writeln(out9, 'vessel min(y) vessel  x');
      end;
    if p='b' then
      begin
        writeln(out10,'vessel min(y) vessel  x');
        writeln(out9, 'vessel min(y) vessel  x');
      end;
    if p='t' then
      begin
        writeln(out10,'vessel min(y) vessel   x');
        writeln(out9, 'vessel min(y) vessel   x');
      end;
    l:=0;
    repeat
    l:=l+1;
    detect_arterialwall_horizontal(x569,y569,vw,minv5,maxv5);
    dy5:=y569-minv5;
    for j:=1 to maxv5-minv5-1+4 do
      begin
        imagepos := 512+ 4*x569 + 341*(minv5-2+j)*4 ;
            seek(infile,imagepos);
            blockread(infile,read569[j],4);
      end;
    min569:=dy5;
```

```
for i:=dy5 to dy5+3 do
 begin
  if read569[min569]>read569[i+1] then
  min569:=i+1;
 end;
y569:=y569+min569-2-dy5;
y600:=y569-dy;
for i:= 1 to 7 do
 begin
  imagepos := 512+ 4*x600 + 341*(y600-5+i)*4 ;
  seek(infile,imagepos);
  blockread(infile,read600[i],4);
 end;
min600:=3;
for i:=4 to 5  do
     if read600[min600]>read600[i] then
  min600:=i;
y600:=y600-4+min600;
avg569[l]:=read569[min569];
avg600[l]:=read600[min600];
sum4:=0;
sum3:=0;
evlsum5:=0;
evlsum6:=0;
evrsum5:=0;
evrsum6:=0;
for s:=1 to 3 do
     for t:= 1 to 3 do
  begin
       imagepos := 512+ 4*(x569-2+s) + 341*(y569-2+t)*4;
       seek(infile,imagepos);
       blockread(infile,sample569[s,t],4);
   imagepos := 512+ 4*(x600-2+s) + 341*(y600-2+t)*4;
   seek(infile,imagepos);
   blockread(infile,sample600[s,t],4);
       if p='t' then
    begin
     imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s-offset)*4;
          seek(infile,imagepos);
          blockread(infile,ev569[s,t],4);
     imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s-offset)*4;
     seek(infile,imagepos);
     blockread(infile,ev600[s,t],4);
          evlsum5:=evlsum5+ev569[s,t];
          evlsum6:=evlsum6+ev600[s,t];
```

```
                    evsum5:=evlsum5;
                    evsum6:=evlsum6;
                end;
               if p='b' then
              begin
               imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s+offset)*4;
                   seek(infile,imagepos);
                   blockread(infile,ev569[s,t],4);
               imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s+offset)*4;
               seek(infile,imagepos);
               blockread(infile,ev600[s,t],4);
                   evrsum5:=evrsum5+ev569[s,t];
                   evrsum6:=evrsum6+ev600[s,t];
                       evsum5:=evrsum5;
                       evsum6:=evrsum6;
                  end;
             if p='B' then
              begin
               imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s-offset)*4;
                   seek(infile,imagepos);
                   blockread(infile,ev569[s,t],4);
               imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s-offset)*4;
               seek(infile,imagepos);
               blockread(infile,ev600[s,t],4);
                       evlsum5:=evlsum5+ev569[s,t];
                   evlsum6:=evlsum6+ev600[s,t];
                   imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s+offset)*4;
                   seek(infile,imagepos);
                   blockread(infile,ev569[s,t],4);
               imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s+offset)*4;
               seek(infile,imagepos);
               blockread(infile,ev600[s,t],4);
                   evrsum5:=evrsum5+ev569[s,t];
                   evrsum6:=evrsum6+ev600[s,t];
                       evsum5:=(evrsum5+evlsum5) div 2;
                       evsum6:=(evrsum6+evlsum6) div 2 ;
              end;
             sum4:=sum4+sample600[s,t];
             sum3:=sum3+sample569[s,t];
            end;
          temp5[l]:=sum3 div 9;
          temp6[l]:=sum4 div 9;
          ev5[l]:=evsum5 div 9;
          ev6[l]:=evsum6 div 9;
          if p='b' then
```

```
          begin
            writeln(out10,minv5:4,y569:8,maxv5:8,x569:8);
            writeln(out9,(minv5-dy):4,y600:8,(maxv5-dy):8,x600:8);
          end;
        if p='t' then
          begin
            writeln(out10,minv5:8,y569:8,maxv5:8,x569:8);
            writeln(out9,(minv5-dy):8,y600:8,(maxv5-dy):8,x600:8);
          end;
        if p='B' then
          begin
            writeln(out10,minv5:8,y569:8,maxv5:8,x569:8);
            writeln(out9,(minv5-dy):8,y600:8,(maxv5-dy):8,x600:8);
          end;
        x569:=x569+1;
        x600:=x600+1;
        until l=scanlength;
        for s:=1 to scanlength do
          begin
            avg3[s]:=temp5[s];
            avg4[s]:=temp6[s];
            evavg5[s]:=ev5[s];
            evavg6[s]:=ev6[s];
            minsum5[s]:=avg569[s];
            minsum6[s]:=avg600[s];
          end;
        close(out9);
        close(out10);
        close(infile);

    end; {scanartery_horizontal}

    procedure scanvein_vertical(var infile:file;var
    x600,x569,y600,y569,scanlength:longint;p:char;
    vw,offset:integer;var avg3,avg4,evavg5,evavg6:ves_array;var result:text);

    var
      dx5,dx6:shortint;
      j,l,i,min569,min600,s,t:integer;
      imagepos,evlsum5,evlsum6,evrsum5,evrsum6,evsum5,evsum6,sum3,sum4,
      minv6,maxv6,minv5,maxv5 :longint;
      read600,read569,avg569,avg600,temp5,temp6,ev5,ev6: array[1..341] of word;
      sample569,sample600,ev600,ev569: array[1..3,1..3] of word;
      next569,next600: array[1..3] of word;
      out9,out10:text;
```

```
procedure detect_venouswall_vertical(x569,x600,y569,y600:longint;vw:integer;var
minv5,maxv5,minv6,maxv6:longint;
var infile:file);
var
  sobely,sobelx: array[1..3,1..3] of integer;
  es5,es6,imagepos:longint;
  test569,read569,test600,read600:array[1..3,1..341] of word;
  detect5,detect6:array[1..341] of integer;

i,j,k,gx5,gy5,gx6,gy6,max569,min569,min5,max5,min6,max6,min600,max600:intege
r;

begin{detect_venouswall_vertical}
   sobely[1,1]:=-1; sobely[1,2]:=0; sobely[1,3]:=1;
   sobely[2,1]:=-2; sobely[2,2]:=0; sobely[2,3]:=2;
   sobely[3,1]:=-1; sobely[3,2]:=0; sobely[3,3]:=1;
   sobelx[1,1]:=-1; sobelx[1,2]:=-2; sobelx[1,3]:=-1;
   sobelx[2,1]:=0; sobelx[2,2]:=0; sobelx[2,3]:=0;
   sobelx[3,1]:=1; sobelx[3,2]:=2; sobelx[3,3]:=1;

   for i:=1 to 3 do
    begin
     for k:= 1 to vw*2+5 do
      begin
       imagepos:= 512+ 4*(x569-vw-3+k) + 341*(y569-2+i)*4  ;
       seek(infile,imagepos);
       blockread(infile,read569[i,k],4);
       imagepos:= 512+ 4*(x600-vw-3+k) + 341*(y600-2+i)*4  ;
       seek(infile,imagepos);

blockread(infile,read600[i,k],4);
      end;
    end;
   for k:= 2 to vw*2+4 do
    begin

gy5:=(read569[1,k-1]*sobely[1,1]+read569[1,k]*sobely[1,2]+read569[1,k+1]*sobely[
1,3]+read569[2,k-1]*sobely[2,1]+read569[2,k]*sobely[2,2]+read569[2,k+1]*sobely[2
,3]+read569[3,k-1]*sobely[3,1]+read569[3,k]*sobely[3,2]+read569[3,k+1]*sobely[3,
3]);

gx5:=(read569[1,k-1]*sobelx[1,1]+read569[1,k]*sobelx[1,2]+read569[1,k+1]*sobelx
[1,3]+read569[2,k-1]*sobelx[2,1]+read569[2,k]*sobelx[2,2]+read569[2,k+1]*sobelx[
2,3]+read569[3,k-1]*sobelx[3,1]+read569[3,k]*sobelx[3,2]+read569[3,k+1]*sobelx[
3,3]);
```

```
        detect5[k]:=(gx5+gy5) div 9;

    gy6:=(read600[1,k-1]*sobely[1,1]+read600[1,k]*sobely[1,2]+read600[1,k+1]*sobely[
    1,3]+read600[2,k-1]*sobely[2,1]+read600[2,k]*sobely[2,2]+read600[2,k+1]*sobely[2
    ,3]+read600[3,k-1]*sobely[3,1]+read600[3,k]*sobely[3,2]+read600[3,k+1]*sobely[3,
    3]);

    gx6:=(read600[1,k-1]*sobelx[1,1]+read600[1,k]*sobelx[1,2]+read600[1,k+1]*sobelx
    [1,3]+read600[2,k-1]*sobelx[2,1]+read600[2,k]*sobelx[2,2]+read600[2,k+1]*sobelx[
    2,3]+read600[3,k-1]*sobelx[3,1]+read600[3,k]*sobelx[3,2]+read600[3,k+1]*sobelx[
    3,3]);

        detect6[k]:=(gx6+gy6) div 9;
          end;
      min5:=maxint;
      max5:=-maxint;
      min569:=x569+vw;
      max569:=x569-vw;
      min6:=maxint;
      max6:=-maxint;
      min600:=x600+vw;
      max600:=x600-vw;
      for k:=3 to vw+8 do
       begin
            if detect5[k] < min5 then
         begin
          min569:=x569-vw-3+k;
          min5:=detect5[k];
         end;
        if detect6[k] < min6 then
         begin
          min600:=x600-vw-3+k;
          min6:=detect6[k];
         end;
          end;
      for k:=vw*2+4 downto vw+2 do
       if detect5[k] > max5 then
        begin
            max5:=detect5[k];
            max569:=x569-vw-3+k;
        end;
      for k:=vw*2+4 downto vw+2 do
       if detect6[k] > max6 then
        begin
            max600:=x600-vw-3+k;
```

```
            max6:=detect6[k];
          end;
        minv5:=min569;
        maxv5:=max569;
        minv6:=min600;
        maxv6:=max600;

      end;{detect_venouswall_vertical}

      begin {scanvein_vertical}
        assign(out9,'vvline6.dat');
        rewrite(out9);
        assign(out10,'vvline5.dat');
        rewrite(out10);
        if p= 'b' then
         begin
          writeln(out10,'vessel min(x) vessel  y');
          writeln(out9, 'vessel min(x) vessel  y');
         end;
        if p='r' then
         begin
          writeln(out10,'vessel min(x) vessel  y');
          writeln(out9, 'vessel min(x) vessel  y');
         end;
        if p='l' then
         begin
          writeln(out10,'vessel min(x) vessel   y');
          writeln(out9, 'vessel min(x) vessel   y');
         end;
        l:=0;
        repeat
        l:=l+1;

      detect_venouswall_vertical(x569,x600,y569,y600,vw,minv5,maxv5,minv6,maxv6,inf
      ile);
        dx5:=x569-minv5; dx6:=x600-minv6;
        for j:=1 to maxv5-minv5+vw*2+1 do
         begin
          imagepos := 512+ 4*(minv5+j-vw-1) + 341*y569*4 ;
          seek(infile,imagepos);
              blockread(infile,read569[j],4);
         end;
        for j:=1 to maxv6-minv6+vw*2+1 do
         begin
          imagepos := 512+ 4*(minv6+j-vw-1) + 341*y600*4 ;
```

```
            seek(infile,imagepos);
            blockread(infile,read600[j],4);
        end;
      min569:=vw-2+dx5;
      for i:=vw-2+dx5 to vw+dx5+3 do
        begin
          if read569[min569]>read569[i+1] then
            min569:=i+1;
        end;
      min600:=vw-2+dx6;
      for i:=vw-2+dx6 to vw+dx6+3 do
        begin
          if read600[min600]>read600[i+1] then
            min600:=i+1;
        end;
      x569:=x569+min569-vw-1-dx5;
      x600:=x600+min600-vw-1-dx6;
      avg569[l]:=read569[min569];
      avg600[l]:=read600[min600];
      sum4:=0;
      sum3:=0;
      evlsum5:=0;
      evlsum6:=0;
      evrsum5:=0;
      evrsum6:=0;
      for s:=1 to 3 do
          for t:= 1 to 3 do
        begin
            imagepos := 512+ 4*(x569-2+s) + 341*(y569-2+t)*4;
            seek(infile,imagepos);
            blockread(infile,sample569[s,t],4);
        imagepos := 512+ 4*(x600-2+s) + 341*(y600-2+t)*4;
        seek(infile,imagepos);
        blockread(infile,sample600[s,t],4);
            if p='l' then
          begin
            imagepos := 512+ 4*(x569-2+s-offset) + 341*(y569-2+t)*4;
                seek(infile,imagepos);
                blockread(infile,ev569[s,t],4);
            imagepos := 512+ 4*(x600-2+s-offset) + 341*(y600-2+t)*4;
            seek(infile,imagepos);
            blockread(infile,ev600[s,t],4);
                evlsum5:=evlsum5+ev569[s,t];
                evlsum6:=evlsum6+ev600[s,t];
                    evsum5:=evlsum5;
```

```
                    evsum6:=evlsum6;
                  end;
                 if p='r' then
               begin
                 imagepos := 512+ 4*(x569-2+s+offset) + 341*(y569-2+t)*4;
                    seek(infile,imagepos);
                    blockread(infile,ev569[s,t],4);
                 imagepos := 512+ 4*(x600-2+s+offset) + 341*(y600-2+t)*4;
                 seek(infile,imagepos);
                 blockread(infile,ev600[s,t],4);
                    evrsum5:=evrsum5+ev569[s,t];
                    evrsum6:=evrsum6+ev600[s,t];
                       evsum5:=evrsum5;
                       evsum6:=evrsum6;
                  end;
              if p='b' then
               begin
                 imagepos := 512+ 4*(x569-2+s-offset) + 341*(y569-2+t)*4;
                    seek(infile,imagepos);
                    blockread(infile,ev569[s,t],4);
                 imagepos := 512+ 4*(x600-2+s-offset) + 341*(y600-2+t)*4;
                 seek(infile,imagepos);
                 blockread(infile,ev600[s,t],4);
                       evlsum5:=evlsum5+ev569[s,t];
                    evlsum6:=evlsum6+ev600[s,t];
                    imagepos := 512+ 4*(x569-2+s+offset) + 341*(y569-2+t)*4;
                    seek(infile,imagepos);
                    blockread(infile,ev569[s,t],4);
                 imagepos := 512+ 4*(x600-2+s+offset) + 341*(y600-2+t)*4;
                 seek(infile,imagepos);
                 blockread(infile,ev600[s,t],4);
                    evrsum5:=evrsum5+ev569[s,t];
                    evrsum6:=evrsum6+ev600[s,t];
                       evsum5:=(evrsum5+evlsum5) div 2;
                       evsum6:=(evrsum6+evlsum6) div 2 ;
               end;
              sum4:=sum4+sample600[s,t];
              sum3:=sum3+sample569[s,t];
            end;
          temp5[l]:=sum3 div 9;
          temp6[l]:=sum4 div 9;
          ev5[l]:=evsum5 div 9;
          ev6[l]:=evsum6 div 9;
          if p='r' then
           begin
```

```
          writeln(out10,minv5:4,x569:8,maxv5:8,y569:8);
          writeln(out9,minv6:4,x600:8,maxv6:8,y600:8);
         end;
        if p='l' then
         begin
          writeln(out10,minv5:8,x569:8,maxv5:8,y569:8);
          writeln(out9,minv6:8,x600:8,maxv6:8,y600:8);
         end;
        if p='b' then
         begin
          writeln(out10,minv5:8,x569:8,maxv5:8,y569:8);
          writeln(out9,minv6:8,x600:8,maxv6:8,y600:8);
         end;
        y569:=y569+1;
        y600:=y600+1;
        until l=scanlength;
        for s:=1 to scanlength do
         begin
          avg3[s]:=temp5[s];
          avg4[s]:=temp6[s];
          evavg5[s]:=ev5[s];
          evavg6[s]:=ev6[s];
         end;
        close(out9);
        close(out10);
        close(infile);

      end; {scanvein_vertical}

      procedure scanvein_horizontal(var infile:file;var
      x600,x569,y600,y569,scanlength:longint;p:char;
      vw,offset:integer;var avg3,avg4,evavg5,evavg6:ves_array;var result:text);

      var
       dy5,dy6,j,l,i,min569,min600,s,t:integer;
       evs5,evs6,temp:word;

      imagepos,evlsum6,evrsum5,evrsum6,evsum5,evsum6,sum3,sum4,evlsum5,minv5,ma
      xv5,minv6,maxv6:longint;
       read600,read569,avg569,avg600,temp5,temp6,ev5,ev6: array[1..341] of word;
       sample569,sample600,ev600,ev569: array[1..3,1..3] of word;
       next569,next600: array[1..3] of word;
       out9,out10:text;

      procedure detect_venouswall_horizontal(x569,x600,y569,y600:longint;vw:integer;var
```

```
minv5,maxv5,minv6,maxv6:longint;
var infile:file);

var
  sobely,sobelx: array[1..3,1..3] of integer;
  imagepos:longint;
  read569,read600:array[1..3,1..341] of integer;
  detect5,detect6:array[1..341] of integer;

i,j,k,gx5,gy5,gx6,gy6,max569,min569,min5,max5,min6,max6,min600,max600:intege
r;
  integer;

begin{detect_venouswall_horizontal}
   sobely[1,1]:=-1; sobely[1,2]:=0; sobely[1,3]:=1;
   sobely[2,1]:=-2; sobely[2,2]:=0; sobely[2,3]:=2;
   sobely[3,1]:=-1; sobely[3,2]:=0; sobely[3,3]:=1;
   sobelx[1,1]:=-1; sobelx[1,2]:=-2; sobelx[1,3]:=-1;
   sobelx[2,1]:=0; sobelx[2,2]:=0; sobelx[2,3]:=0;
   sobelx[3,1]:=1; sobelx[3,2]:=2; sobelx[3,3]:=1;
   for i:=1 to 3 do
    begin
     for k:= 1 to vw*2+5 do
      begin
       imagepos:= 512+ 4*(x569-2+i) + 341*(y569-vw-3+k)*4  ;
       seek(infile,imagepos);
       blockread(infile,read569[i,k],4);
       imagepos:= 512+ 4*(x600-2+1) + 341*(y600-vw-3+k)*4  ;
       seek(infile,imagepos);
       blockread(infile,read600[i,k],4);
      end;
    end;
   for k:= 2 to vw*2+4 do
    begin

gy5:=(read569[1,k-1]*sobely[1,1]+read569[1,k]*sobely[1,2]+read569[1,k+1]*sobely[
1,3]+read569[2,k-1]*sobely[2,1]+read569[2,k]*sobely[2,2]+read569[2,k+1]*sobely[2
,3]+read569[3,k-1]*sobely[3,1]+read569[3,k]*sobely[3,2]+read569[3,k+1]*sobely[3,
3]);

gx5:=(read569[1,k-1]*sobelx[1,1]+read569[1,k]*sobelx[1,2]+read569[1,k+1]*sobelx
[1,3]+read569[2,k-1]*sobelx[2,1]+read569[2,k]*sobelx[2,2]+read569[2,k+1]*sobelx[
2,3]+read569[3,k-1]*sobelx[3,1]+read569[3,k]*sobelx[3,2]+read569[3,k+1]*sobelx[
3,3]);
```

```
        detect5[k]:=(gx5+gy5) div 9;

    gy6:=(read600[1,k-1]*sobely[1,1]+read600[1,k]*sobely[1,2]+read600[1,k+1]*sobely[
    1,3]+read600[2,k-1]*sobely[2,1]+read600[2,k]*sobely[2,2]+read600[2,k+1]*sobely[2
    ,3]+read600[3,k-1]*sobely[3,1]+read600[3,k]*sobely[3,2]+read600[3,k+1]*sobely[3,
    3]);


    gx6:=(read600[1,k-1]*sobelx[1,1]+read600[1,k]*sobelx[1,2]+read600[1,k+1]*sobelx
    [1,3]+read600[2,k-1]*sobelx[2,1]+read600[2,k]*sobelx[2,2]+read600[2,k+1]*sobelx[
    2,3]+read600[3,k-1]*sobelx[3,1]+read600[3,k]*sobelx[3,2]+read600[3,k+1]*sobelx[
    3,3]);

        detect6[k]:=(gx6+gy6) div 9;
          end;
      min5:=maxint;
      max5:=-maxint;
      min569:=y569+vw-1;
      max569:=y569-vw+1;
      min6:=maxint;
      max6:=-maxint;
      min600:=y600+vw-1;
      max600:=y600-vw+1;
      for k:=3 to vw+8 do
       begin
            if detect5[k] < min5 then
         begin
          min569:=y569-vw-3+k;
          min5:=detect5[k];
         end;
        if detect6[k] < min6 then
         begin
          min600:=y600-vw-3+k;
          min6:=detect6[k];
         end;
          end;
      for k:=vw*2+4 downto vw+2 do
       if detect5[k] > max5 then
        begin
             max5:=detect5[k];
             max569:=y569-vw-3+k;
        end;
      for k:=vw*2+4 downto vw+2 do
       if detect6[k] > max6 then
        begin
```

```
                max600:=y600-vw-3+k;
              max6:=detect6[k];
            end;
        minv5:=min569;
        maxv5:=max569;
        minv6:=min600;
        maxv6:=max600;
    end;{detect_venouswall_horizontal}

    begin {scanvein_horizontal}
        assign(out9,'hvline6.dat');
        rewrite(out9);
        assign(out10,'hvline5.dat');
        rewrite(out10);
        if p= 'B' then
         begin
          writeln(out10,'vessel  min(y)  vessel   x');
          writeln(out9, 'vessel  min(y)  vessel   x');
         end;
        if p='b' then
         begin
          writeln(out10,'vessel  min(y)  vessel   x');
          writeln(out9, 'vessel  min(y)  vessel   x');
         end;
        if p='t' then
         begin
          writeln(out10,'vessel  min(y)  vessel    x');
          writeln(out9, 'vessel  min(y)  vessel    x');
         end;
        l:=0;
        repeat
        l:=l+1;

    detect_venouswall_horizontal(x569,x600,y569,y600,vw,minv5,maxv5,minv6,maxv6,
    infile);
        dy5:=y569-minv5; dy6:=y600-minv6;
        for j:=1 to maxv5-minv5+vw*2+1 do
         begin
          imagepos := 512+ 4*x569 + 341*(minv5+j-vw-1)*4 ;
          seek(infile,imagepos);
              blockread(infile,read569[j],4);
         end;
        for j:=1 to maxv6-minv6+vw*2+1 do
         begin
              imagepos := 512+ 4*x600 + 341*(minv6+j-vw-1)*4 ;
```

```
        seek(infile,imagepos);
      blockread(infile,read600[j],4);
     end;
    min569:=vw-2+dy5;
    for i:=vw-2+dy5 to vw+dy5+3 do
     begin
      if read569[min569]>read569[i+1] then
      min569:=i+1;
     end;
    min600:=vw-2+dy6;
    for i:=vw-2+dy6 to vw+dy6+3 do
     begin
      if read600[min600]>read600[i+1] then
      min600:=i+1;
     end;
    y569:=y569+min569-vw-1-dy5;
    y600:=y600+min600-vw-1-dy6;
    avg569[l]:=read569[min569];
    avg600[l]:=read600[min600];
    sum4:=0;
    sum3:=0;
    evlsum5:=0;
    evlsum6:=0;
    evrsum5:=0;
    evrsum6:=0;
    for s:=1 to 3 do
        for t:= 1 to 3 do
      begin
          imagepos := 512+ 4*(x569-2+s) + 341*(y569-2+t)*4;
          seek(infile,imagepos);
          blockread(infile,sample569[s,t],4);
       imagepos := 512+ 4*(x600-2+s) + 341*(y600-2+t)*4;
       seek(infile,imagepos);
       blockread(infile,sample600[s,t],4);
       if p='t' then
        begin
         imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s-offset)*4;
             seek(infile,imagepos);
             blockread(infile,ev569[s,t],4);
         imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s-offset)*4;
         seek(infile,imagepos);
         blockread(infile,ev600[s,t],4);
             evlsum5:=evlsum5+ev569[s,t];
             evlsum6:=evlsum6+ev600[s,t];
             evsum5:=evlsum5;
```

```
                evsum6:=evlsum6;
                  end;
                 if p='b' then
               begin
                imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s+offset)*4;
                    seek(infile,imagepos);
                    blockread(infile,ev569[s,t],4);
                imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s+offset)*4;
                seek(infile,imagepos);
                blockread(infile,ev600[s,t],4);
                    evrsum5:=evrsum5+ev569[s,t];
                    evrsum6:=evrsum6+ev600[s,t];
                    evsum5:=evrsum5;
                        evsum6:=evrsum6;
                   end;
              if p='B' then
               begin
                imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s-offset)*4;
                    seek(infile,imagepos);
                    blockread(infile,ev569[s,t],4);
                imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s-offset)*4;
                seek(infile,imagepos);
                blockread(infile,ev600[s,t],4);
                        evlsum5:=evlsum5+ev569[s,t];
                    evlsum6:=evlsum6+ev600[s,t];
                    imagepos := 512+ 4*(x569-2+t) + 341*(y569-2+s+offset)*4;
                    seek(infile,imagepos);
                    blockread(infile,ev569[s,t],4);
                imagepos := 512+ 4*(x600-2+t) + 341*(y600-2+s+offset)*4;
                seek(infile,imagepos);
                blockread(infile,ev600[s,t],4);
                    evrsum5:=evrsum5+ev569[s,t];
                    evrsum6:=evrsum6+ev600[s,t];
                        evsum5:=(evrsum5+evlsum5) div 2;
                        evsum6:=(evrsum6+evlsum6) div 2 ;
                  end;
              sum4:=sum4+sample600[s,t];
              sum3:=sum3+sample569[s,t];
             end;
           temp5[l]:=sum3 div 9;
           temp6[l]:=sum4 div 9;
           ev5[l]:=evsum5 div 9;
           ev6[l]:=evsum6 div 9;
           if p='b' then
            begin
```

```
            writeln(out10,minv5:4,y569:8,maxv5:8,x569:8);
            writeln(out9,minv6:4,y600:8,maxv6:8,x600:8);
           end;
          if p='t' then
           begin
            writeln(out10,minv5:8,y569:8,maxv5:8,x569:8);
            writeln(out9,minv6:8,y600:8,maxv6:8,x600:8);
           end;
          if p='B' then
           begin
            writeln(out10,minv5:8,y569:8,maxv5:8,x569:8);
            writeln(out9,minv6:8,y600:8,maxv6:8,x600:8);
           end;
          x569:=x569+1;
          x600:=x600+1;
          until l=scanlength;
          for s:=1 to scanlength do
           begin
            avg3[s]:=temp5[s];
            avg4[s]:=temp6[s];
            evavg5[s]:=ev5[s];
            evavg6[s]:=ev6[s];
           end;
          close(out9);
          close(out10);
          close(infile);

        end; {scanvein_horizontal}

        procedure
        competed(evavg5,evavg6,avg3,avg4,minsum5,minsum6:ves_array;scanlength:longint;
        di:integer;
        var evi569,evi600:longint;var od569,od600,r,pr,pod569:real);

        var
         kernsum5,kernsum6,evtot5,evtot6,msum5,msum6:longint;
         i:integer;

        begin{compute_od}
          kernsum5:=0;
          kernsum6:=0;
          evtot5:=0;
          evtot6:=0;
          msum5:=0; msum6:=0;
          for i:=1 to scanlength do
```

```
            begin
              kernsum5:=kernsum5+avg3[i];
              kernsum6:=kernsum6+avg4[i];
                  evtot5:=evtot5+evavg5[i];
                  evtot6:=evtot6+evavg6[i];
              msum5:=msum5+minsum5[i];
              msum6:=msum6+minsum6[i];
            end;
          evi569:=evtot5 div scanlength;
          evi600:=evtot6 div scanlength;
          evi569:=evi569-di;
          evi600:=evi600-di;
          vi569:=kernsum5 div scanlength;
          vi600:=kernsum6 div scanlength;
          vi569:=vi569-di;
          vi600:=vi600-di;
          od600:=ln(evi600/vi600)/2.303;
          od569:=ln(evi569/vi569)/2.303;
          r:=od600/od569;
          pod569:=ln((evi600*1.5)/vi569)/2.303;
          pr:=od600/pod569;

        end;{competed}

        procedure
        print(vi600,vi569,evi569,evi600:longint;di:integer;od569,od600,r,pr,pod569:real;
        var result:text);
        var
          pod569u,pru:real;

        begin{print}
          writeln('Absolute IV600 , EV600 = ',vi600+di,', ',evi600+di);
          writeln(result,'Absolute IV600 , EV600 = ',vi600+di,', ',evi600+di);
          writeln('Dark current subtracted IV600 , EV600 = ',vi600,', ',evi600);
          writeln(result,'Dark current subtracted IV600 , EV600 = ',vi600,', ',evi600);
          writeln('OD600 = ',od600:5:4);
          writeln(result,'OD600 = ',od600:5:4);
          writeln('Absolute IV569 , EV569 = ',vi569+di,', ',evi569+di);
          writeln(result,'Absolute IV569 , EV569 = ',vi569+di,', ',evi569+di);
          writeln('Dark current subtracted IV569 , EV569 = ',vi569,', ',evi569);
          writeln(result,'Dark current subtracted IV569 , EV569 = ',vi569,', ',evi569);
          writeln('OD569 = ',od569:5:4);
          writeln(result,'OD569 = ',od569:5:4);
          writeln('ODR = ',r:5:4);
          writeln(result,'ODR = ',r:5:4);
```

```
    writeln(result);
    writeln(result,'Pseudo OD569 = ', pod569:5:4);
    writeln(result,'Peludo ODR = ', pr:5:4);
    writeln('Pseudo OD569 = ', pod569:5:4);
    writeln('Peludo ODR = ', pr:5:4);
    pod569u:=ln(evi600/vi569)/2.303;
    Pau:=od600/pod569u;
    writeln(result);
    writeln;
    close(result);
end;{print}

Begin{main}
    vein:=true;
    getinput(x600,x569,y600,y569,dx,dy,scanlength,p,direction,vw,offset,vein);
    init_textfiles(result,infile,outfile,direction,vein);
    if vein=false then
     begin
      if direction = 'v' then

scanartery_vertical(infile,x600,x569,y600,y569,dx,scanlength,p,vw,offset,avg3,
        avg4,evavg5,evavg6,minsum5,minsum6,result);
      if direction = 'h' then

scanartery_horizontal(infile,x600,x569,y600,y569,dy,scanlength,p,vw,offset,avg3,
        avg4,evavg5,evavg6,minsum5,minsum6,result);
     end;
    if vein = true then
     begin
      if direction = 'v' then
       scanvein_vertical(infile,x600,x569,y600,y569,scanlength,p,vw,offset,
       avg3,avg4,evavg5,evavg6,result);
      if direction = 'h' then
       scanvein_horizontal(infile,x600,x569,y600,y569,scanlength,p,vw,offset,
       avg3,avg4,evavg5,evavg6,result);
     end;

competed(evavg5,evavg6,avg3,avg4,minsum5,minsum6,scanlength,di,evi569,evi600,
od569,od600,r,pr,pod569);
    print(vi600,vi569,evi569,evi600,di,od569,od600,r,pr,pod569,result);
end.
```

What is claimed is:

1. An apparatus for evaluating oxygen utilization in posterior pole tissue of an eye, said apparatus comprising:

a fundus camera capable of generating an intermediate image of blood vessels in an eye;

a beam splitter assembly comprising a beam splitter, a first bandpass filter and a second bandpass filter, said beam splitter being capable of splitting said intermediate image into a first image and a second image; and

an electronic imaging device capable of electronically recording said first image and said second image after said first and second images have passed through said first bandpass filter and said second bandpass filter;

said first bandpass filter having a first wavelength and said second bandpass filter having a second wavelength, said first and second wavelengths being selected to optimize the imaging capability of said electronic imaging device with respect to blood oxygen saturation.

2. The apparatus of claim 1, wherein said electronic imaging device comprises a digital imaging device.

3. The apparatus of claim 2, wherein said electronic imaging device comprises a digital charge-coupled device.

4. The apparatus of claim 1, wherein said first wavelength of said first bandpass filter is 600 nm ± 2.5 nm.

5. The apparatus of claim 4, wherein said second wavelength of said second bandpass filter is 569 nm ± 2.5 nm.

6. The apparatus of claim 4, wherein said second wavelength of said second bandpass filter is 586 nm ± 2.5 nm.

7. The apparatus of claim 4, wherein said second wavelength of said second bandpass filter is 558 nm ± 2.5 nm.

8. The apparatus of claim 1, wherein said fundus camera has a body and an imaging plane, said imaging plane being external to said body of said fundus camera.

9. The apparatus of claim 1, wherein said fundus camera has a body and an imaging plane, said imaging plane being internal to said body of said fundus camera.

10. The apparatus of claim 9, wherein said fundus camera further comprises a filter wheel fitted with a -6 diopter lens to form said imaging plane internal to said body of said fundus camera.

11. The apparatus of claim 1, wherein said beam splitter assembly further comprises a plurality of front surface mirrors to position said first and second images relative to each other.

12. The apparatus of claim 11, wherein said at least one of said plurality of front surface mirrors is adjustable.

13. The apparatus of claim 12, wherein said beam splitter assembly further comprises means for mounting said plurality of front surface mirrors in said beam splitter assembly.

14. The apparatus of claim 13, wherein said means for mounting said plurality of front surface mirrors in said beam splitter assembly comprises:

a base plate, said base plate having a first side portion having a first plurality of rails, and a second side portion having a second plurality of rails;

a first plurality of mirror mounts disposed on said first plurality of rails and a second plurality of mirror mounts disposed on said second plurality of rails, a first portion of said plurality of front surface mirrors being attached to said first plurality of mirror mounts and a second portion of said plurality of front surface mirrors being attached to said second plurality of mirror mounts;

a first drive assembly selectively attached to at least one of said first plurality of mirror mounts, wherein movement of said first drive assembly may result in movement of at least one of said first portion of said plurality of front surface mirrors; and

a second drive assembly selectively attached to at least one of said second plurality of mirror mounts, wherein movement of said second drive assembly may result in movement of at least one of said second portion of said plurality of front surface mirrors.

15. The apparatus of claim 14, wherein said first drive assembly comprises a first micrometer and said second drive assembly comprises a second micrometer.

16. The apparatus of claim 15, wherein;

said first plurality of rails consists of two rails and said first plurality of mirror mounts consists of two mirror mounts, one of said mirror mounts holding a first of said plurality of front surface mirrors, and the other of said mirror mounts holding a second of said plurality of front surface mirrors; and

said second plurality of rails consists of two rails and said second plurality of mirror mounts consists of two mirror mounts, one of said mirror mounts holding a third of said plurality of front surface mirrors, and the other of said mirror mounts holding a fourth of said plurality of front surface mirrors.

17. The apparatus of claim 16, further comprising a cover disposed over said beam splitter assembly to protect said beam splitter assembly from contamination.

18. The apparatus of claim 11, said beam splitter assembly having a light entrance end and a light exit end, and further comprising a baffle disposed between said first image and said second image proximal to said light exit end of said beam splitter assembly.

19. The apparatus of claim 1, wherein said fundus camera, said beam splitter and said electronic imaging device comprise an integral unit.

20. A method for evaluating oxygen utilization in posterior pole tissue of an eye, said method comprising:

reflecting a light beam off of blood vessels in an eye to create an intermediate

image of said blood vessels;

splitting said intermediate image into a first image and a second image;

filtering said first image such that said first image has a first wavelength, and filtering said second image such that said second image has a second wavelength; and

producing an electronic recording of said filtered first and second images;

wherein said first wavelength of said first image and said second wavelength of said second image are selected to optimize said electronic recording with respect to blood oxygen saturation.

21. The method of claim 20, further comprising a step for determining average reflected light intensity inside and outside of a first of said blood vessels from said electronically recorded first and second images.

22. The method of claim 21, wherein said step for determining average reflected light intensity inside and outside of said first of said blood vessels from said electronically recorded first and second images comprises:

scanning said electronically recorded first image and said second image; and

separately for said first image and said second image:

identifying a path of minimum reflection inside said first of said blood vessels in said image;

determining said value of average reflected light intensity inside said first of said blood vessels along said path of minimum reflection;

identifying a path of extravascular reflection in said image at a fixed distance from said path of minimum reflection; and

determining said value of average reflected light intensity outside said

first of said blood vessels along said path of extravascular reflection.

23. The method of claim 20, further comprising a step for determining blood oxygen saturation in a vessel using said electronically recorded first and second images and empirical relationships between blood oxygen saturation and optical density.

24. The method of claim 23, further comprising a step for determining a change in said vessel blood oxygen saturation using said electronically recorded first and second images and empirical relationships between blood oxygen saturation and optical density.

25. The method of claim 20, further comprising a step for determining venous blood oxygen saturation using said electronically recorded first and second images and empirical relationships between blood oxygen saturation and optical density.

26. The method of claim 25, wherein said step for determining venous blood oxygen saturation using said electronically recorded first and second images and empirical relationships between blood oxygen saturation and optical density comprises a direct reflectance method.

27. The method of claim 26, wherein said blood vessels comprise an artery, and wherein said step of producing an electronic recording of said filtered first and second images comprises using a conventional instrument to obtain a known value of systemic blood oxygen saturation simultaneously with said production of said

electronic recording, and said step of producing an electronic recording is repeated for a subject for a plurality of values of systemic blood oxygen saturation, and wherein said direct reflectance method comprises:

determining, for said plurality of values of systemic blood oxygen saturation, arterial optical density ratios from data contained in said electronic recording; and

comparing said arterial optical density ratios to said known values of systemic blood oxygen saturation corresponding therewith to determine oxygen sensitivity.

28. The method of claim 27, wherein said step of determining arterial optical density ratios comprises:

determining, for said plurality of values of systemic blood oxygen saturation, average reflected light intensities inside and outside said artery in said first image and second image;

determining, for said plurality of values of systemic blood oxygen saturation, values of arterial optical density for said first image and said second image using said average reflected light intensities; and

determining, for said plurality of values of systemic blood oxygen saturation, said arterial optical density ratios from said values of arterial optical density.

29. The method of claim 25, wherein said step for determining venous blood oxygen saturation using said electronically recorded first and second images and empirical relationships between blood oxygen saturation and optical density comprises a pigmentation correction method.

30. The method of claim 25, wherein said blood vessels comprise an artery, and

wherein said step of producing an electronic recording of said filtered first and second images comprises using a conventional instrument to obtain a known value of systemic blood oxygen saturation simultaneously with said production of said electronic recording, and said step of producing an electronic recording is repeated for a plurality of subjects at a plurality of values of systemic blood oxygen saturation, and wherein said pigmentation correction method comprises:

determining, for said plurality of subjects, arterial optical density ratios from data contained in said electronic recording, corrected for pigmentation effects by assuming that pigment light absorption in said first image is insignificant compared to pigment light absorption in said second image; and

comparing said corrected arterial optical density ratios to said known values of systemic blood oxygen saturation corresponding therewith to determine pigmentation corrected oxygen sensitivity.

31. The method of claim 30, wherein said step of determining arterial optical density ratios corrected for pigmentation effects comprises:

determining, for said plurality of subjects at said plurality of values of systemic blood oxygen saturation, average reflected light intensities inside and outside said artery in said first image and second image;

determining, for said plurality of subjects at said plurality of values of systemic blood oxygen saturation, average reflected light intensity for a dark area in said electronic recording;

determining, for said plurality of subjects at said plurality of values of systemic blood oxygen saturation, vessel optical densities for said artery in said first image, not corrected for pigmentation effects, vessel optical densities for said artery in

said second image, corrected for pigmentation effects by assuming that pigment light absorption in said first image is insignificant compared to pigment light absorption in said second image, and using said average light intensities outside said artery in said first image to correct said average light intensities outside said artery in said second image; and

determining, for said plurality of subjects at said plurality of values of systemic blood oxygen saturation, corrected arterial optical density ratios from said uncorrected arterial optical densities of said vessels in said first image and said corrected arterial optical densities of said vessels in said second image.

32. The method of claim 31, further comprising:

determining, for said plurality of subjects, diameters of said arteries from which data is recorded; and

comparing said corrected arterial optical density ratios to said artery diameters to determine a value of vessel diameter sensitivity.

33. The method of claim 30, wherein said pigmentation correction method comprises using predetermined values of pigmentation-corrected oxygen sensitivity and vessel diameter sensitivity to determine venous blood oxygen saturation corrected for pigmentation effects and blood vessel diameter.

34. The method of claim 25, wherein said step for determining venous blood oxygen saturation using said electronically recorded first and second images and empirical relationships between blood oxygen saturation and optical density comprises an intravascular reflectance method.

35. The method of claim 25, wherein said blood vessels comprise an artery, said eye has a fundus, and said step of producing an electronic recording of said filtered first and second images comprises using a conventional instrument to obtain a known value of systemic blood oxygen saturation simultaneously with said production of said electronic recording, and said step of producing an electronic recording is repeated for a plurality of subjects at a plurality of values of systemic blood oxygen saturation, and wherein said intravascular reflectance method comprises:

determining, for said plurality of subjects at said plurality of values of systemic blood oxygen saturation, pigment-independent arterial optical density ratios assuming said fundus is unpigmented; and

comparing said arterial optical density ratios to said known values of systemic blood oxygen saturation corresponding therewith to determine oxygen sensitivity.

36. The method of claim 35, wherein said step of determining pigment-independent arterial optical density ratios comprises:

determining for each of said subjects, at 100% arterial blood saturation, a first set of average reflected light intensities inside said artery in said first image and said second image;

using a ratio of broadband extinction coefficients for said first image and said second image to determine values of unpigmented reflectance for each of said subjects using said first set of average reflected light intensities;

determining, for said plurality of subjects at said plurality of values of systemic blood oxygen saturation, average reflected light intensities inside said artery in said first image and second image;

determining pigment-independent arterial optical density ratios for said

plurality of subjects at said plurality of values of systemic blood oxygen saturation, using said average reflected light intensities inside said artery in said first image and second image and corresponding values of unpigmented reflectance;

comparing said arterial optical density ratios to said known values of systemic blood oxygen saturation corresponding therewith to determine a value of oxygen sensitivity for each of said plurality of subjects; and

determining a mean value of said values of oxygen sensitivity for said plurality of subjects.

37. The method of claim 36, further comprising:

determining, for said plurality of subjects, diameters of said arteries for which data is recorded; and

comparing arterial optical density ratios to said artery diameters to determine a value of vessel diameter sensitivity.

38. The method of claim 35, wherein said intravascular reflectance method comprises using a predetermined value of mean oxygen sensitivity and vessel diameter sensitivity to determine venous blood oxygen saturation independent of pigmentation effects and corrected for blood vessel diameter.

39. The method of claim 25, wherein said steps for determining venous blood oxygen saturation using said electronically recorded first and second images are automated using a computer, such that said electronically recorded first and second images are scanned, analyzed and venous blood oxygen saturation determined automatically.

40. The method of claim 25, wherein said determination of venous blood oxygen saturation is used to diagnose an eye disease.

41. The method of claim 40, wherein said eye disease is selected from the group consisting of diabetic retinopathy, papallopathy, glaucoma, retinal vein occlusion, optic atrophy and macular degeneration.

42. The method of claim 20, wherein said step of producing an electronic recording comprises using a digital imaging device.

43. The method of claim 42, wherein said step of producing an electronic recording comprises using a digital charge-coupled device.

44. The method of claim 20, wherein said first wavelength of said first bandpass filter is chosen to be 600 nm ± 2.5 nm.

45. The method of claim 20, wherein said second wavelength of said second bandpass filter is chosen to be 569 nm ± 2.5 nm.

46. The apparatus of claim 20, wherein said second wavelength of said second bandpass filter is chosen to be 586 nm ± 2.5 nm.

47. The method of claim 20, wherein said second wavelength of said second bandpass filter is chosen to be 558 nm ± 2.5 nm.

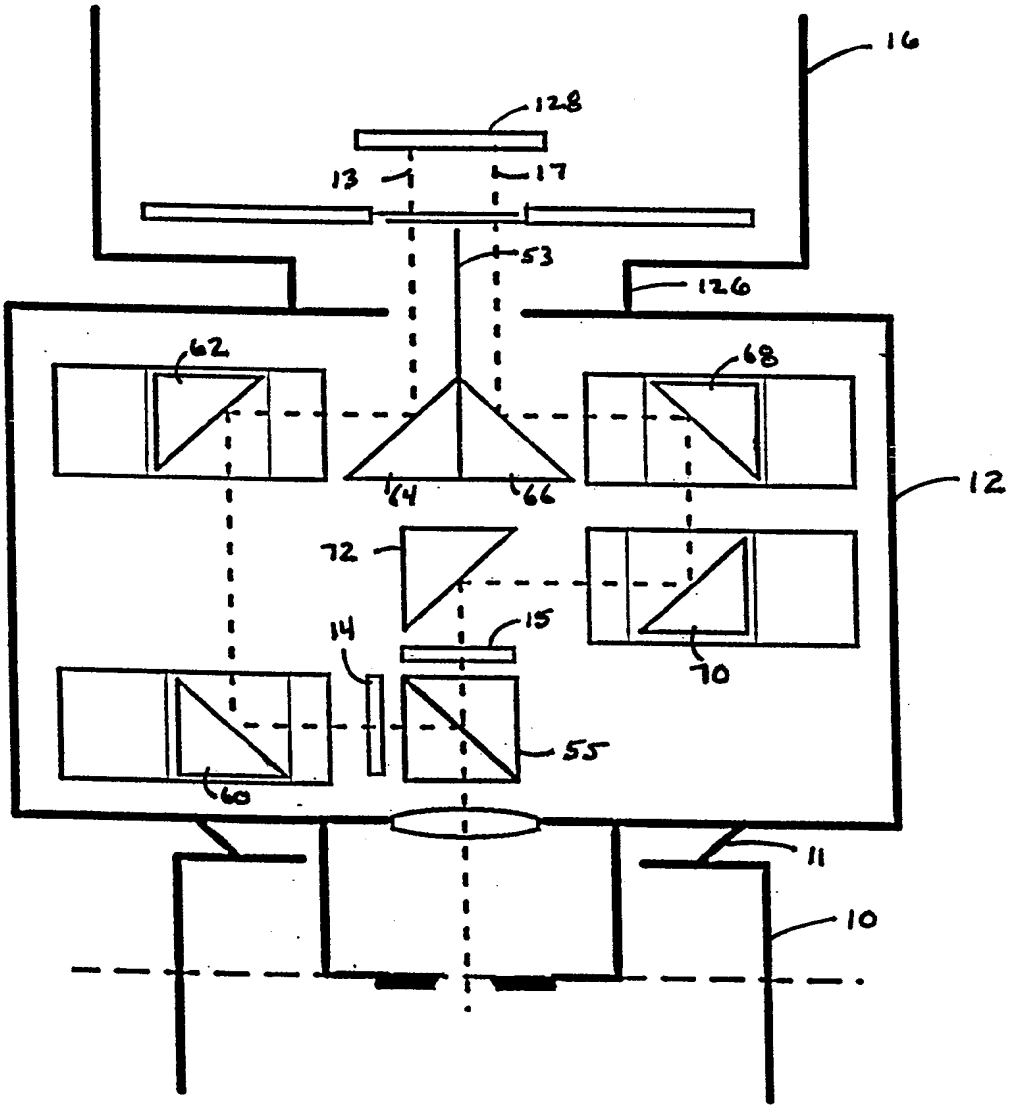

FIGURE 1

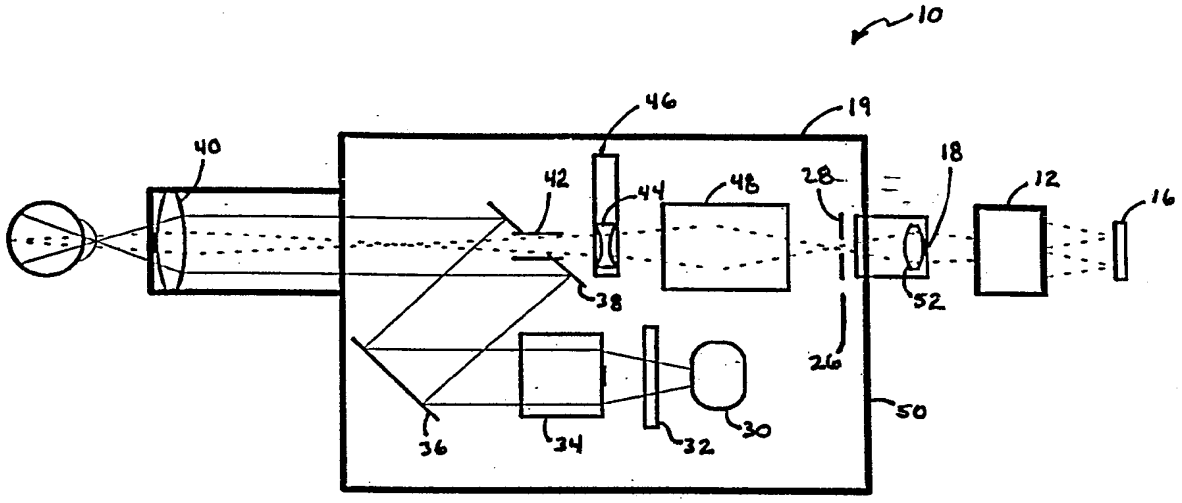

FIG. 2

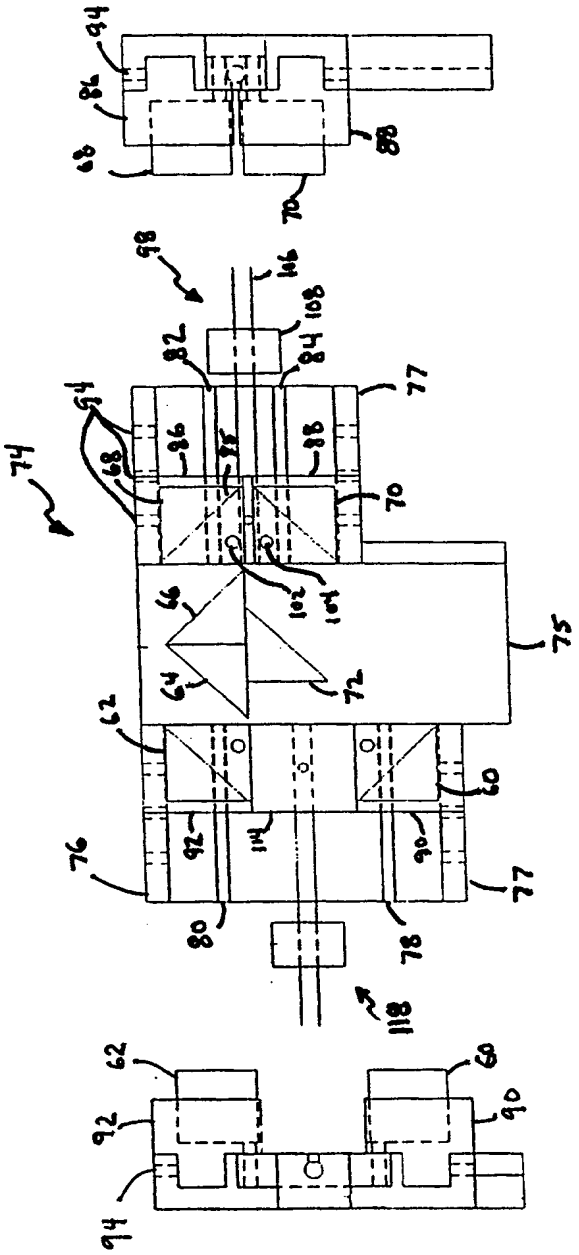

FIG. 3C

FIG. 3A

FIG. 3B

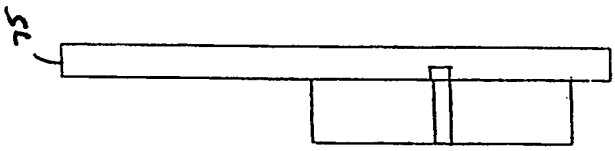

FIG. 4C

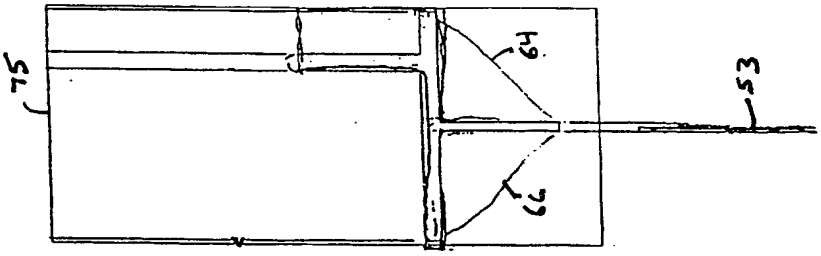

FIG. 4A

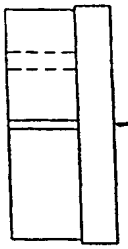

FIG. 4B

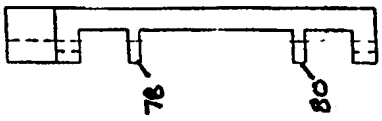

FIG. 5C

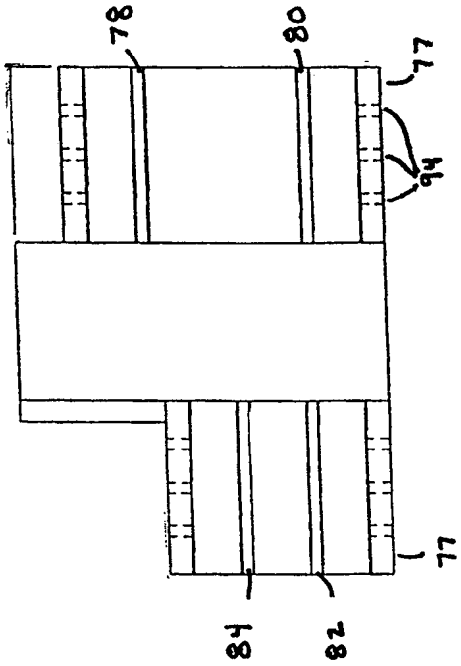

FIG. 5A

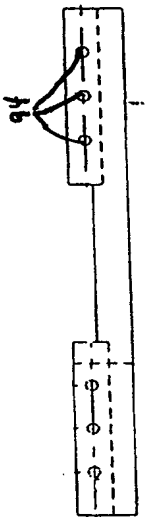

FIG. 5D

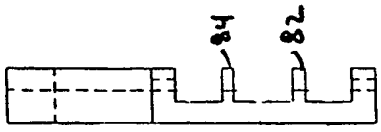

FIG. 5B

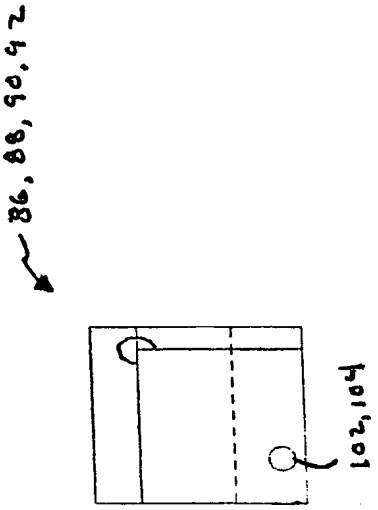

FIG. 6A

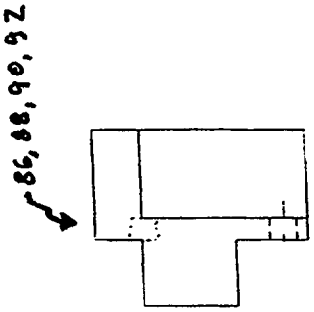

FIG. 6B

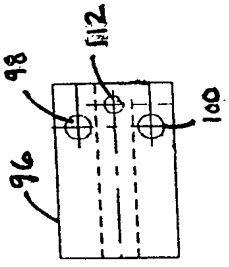

FIG. 7A

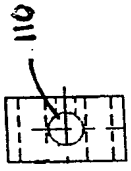

FIG. 7B

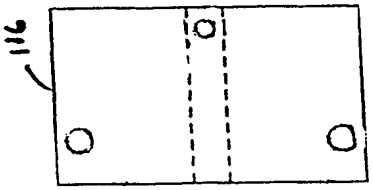

FIG. 8A

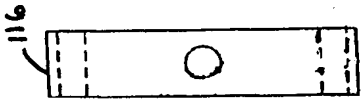

FIG. 8B

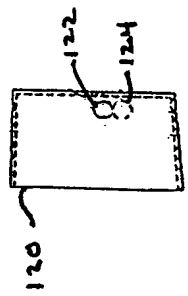

FIG. 9C

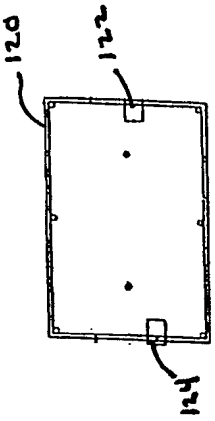

FIG. 9A

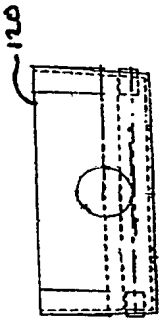

FIG. 9B

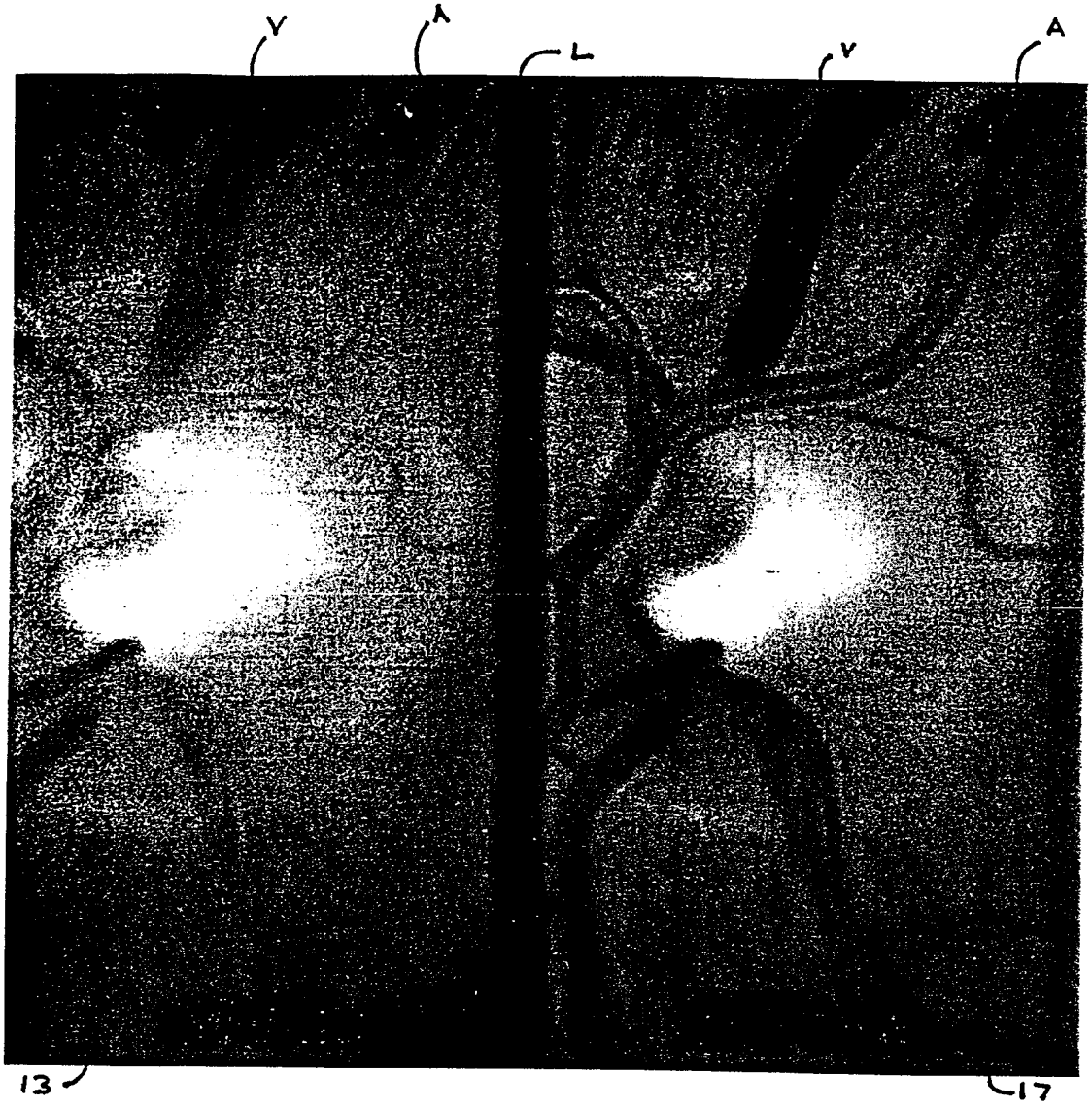

FIG. 10

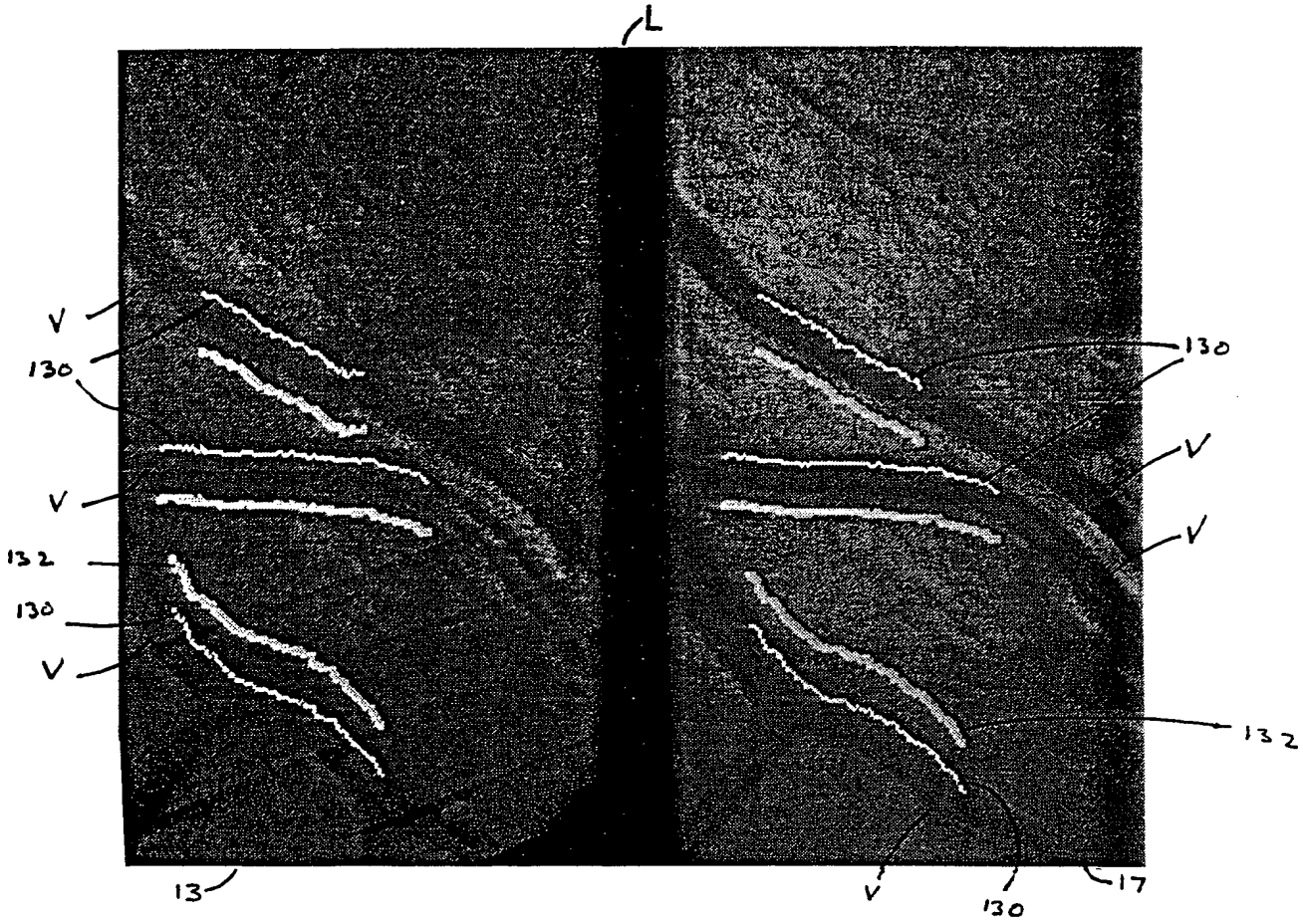

FIG. 11

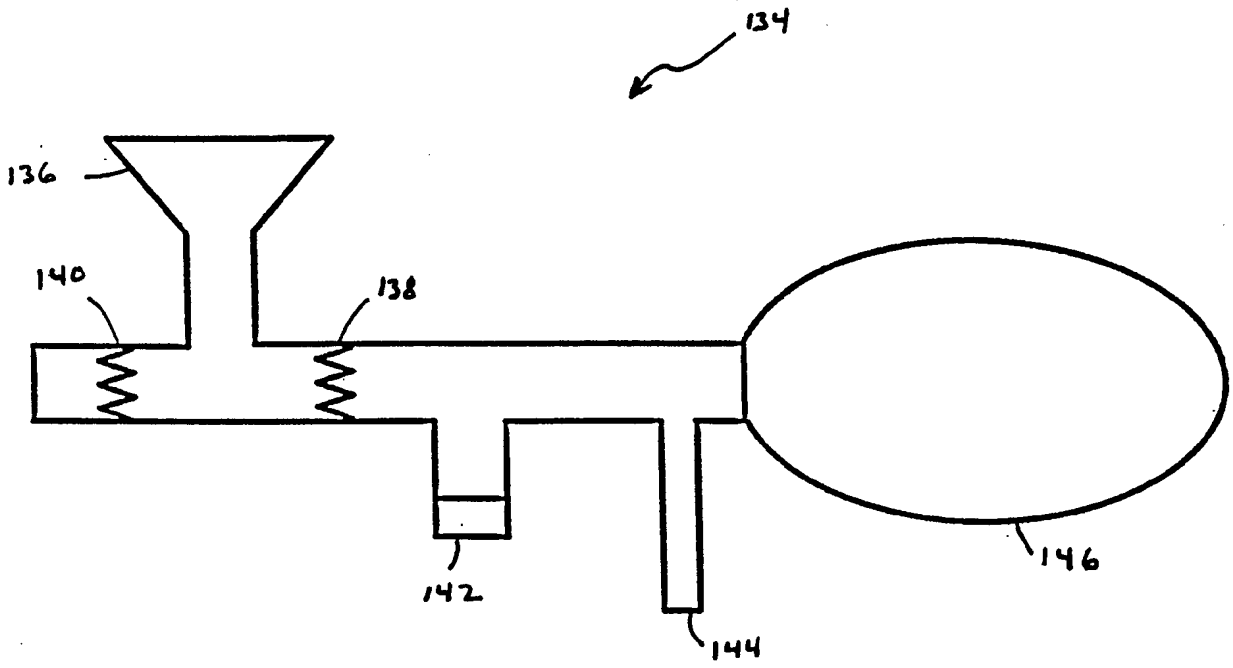

FIG. 12

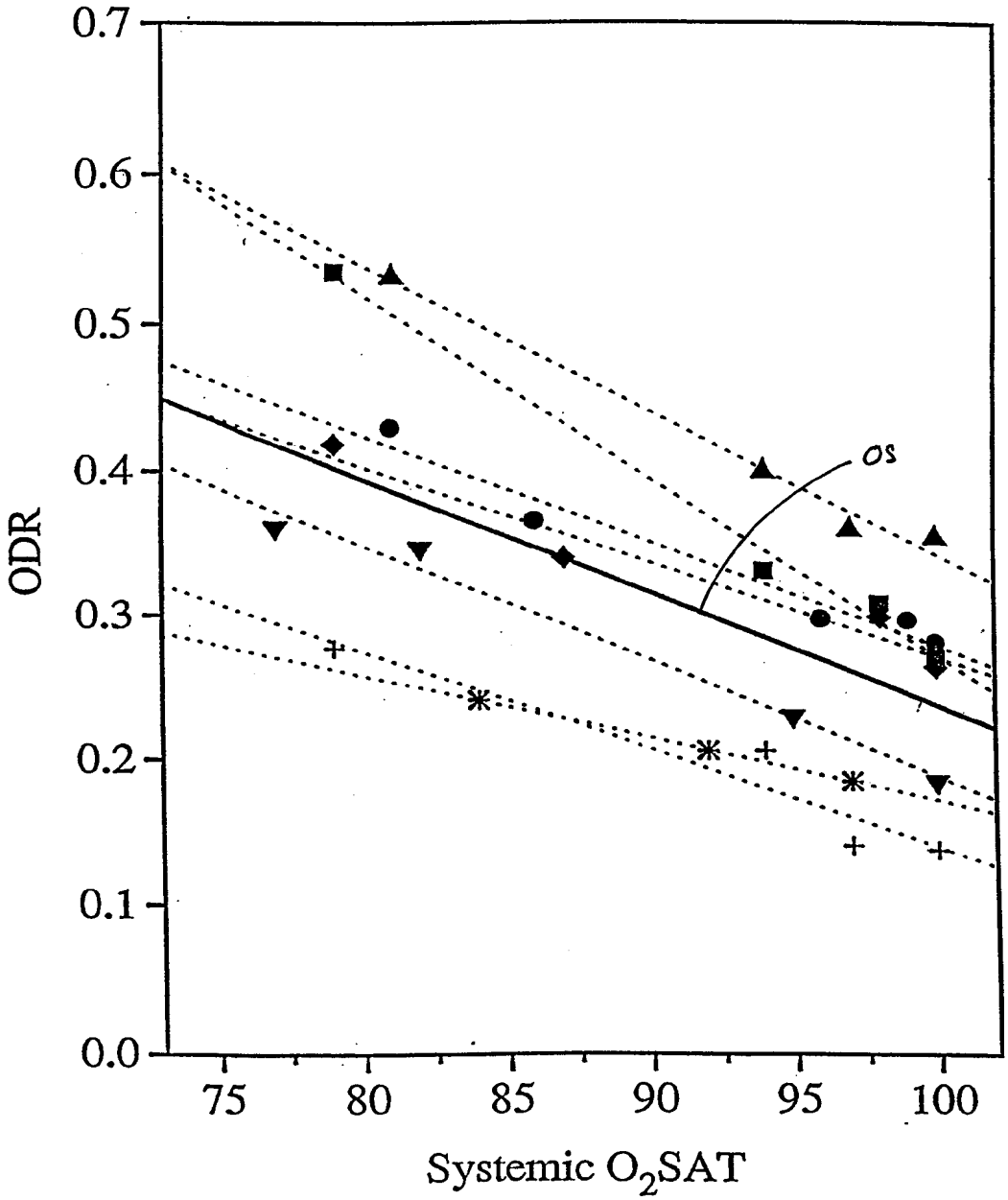

FIG. 13

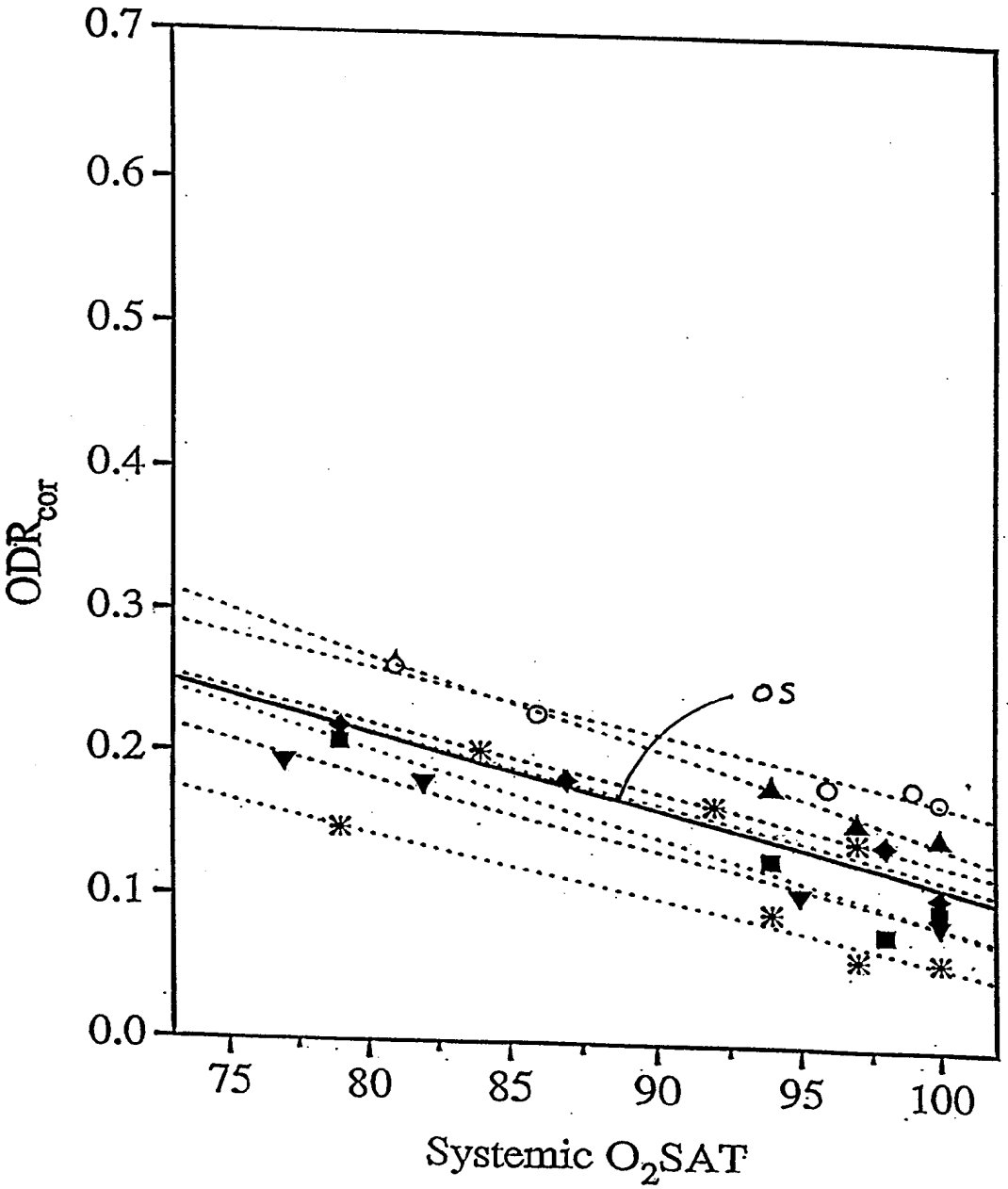
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
ODR$_{cor}$
OS
75
80
85
90
95
100
Systemic O_2SAT

FIG. 14

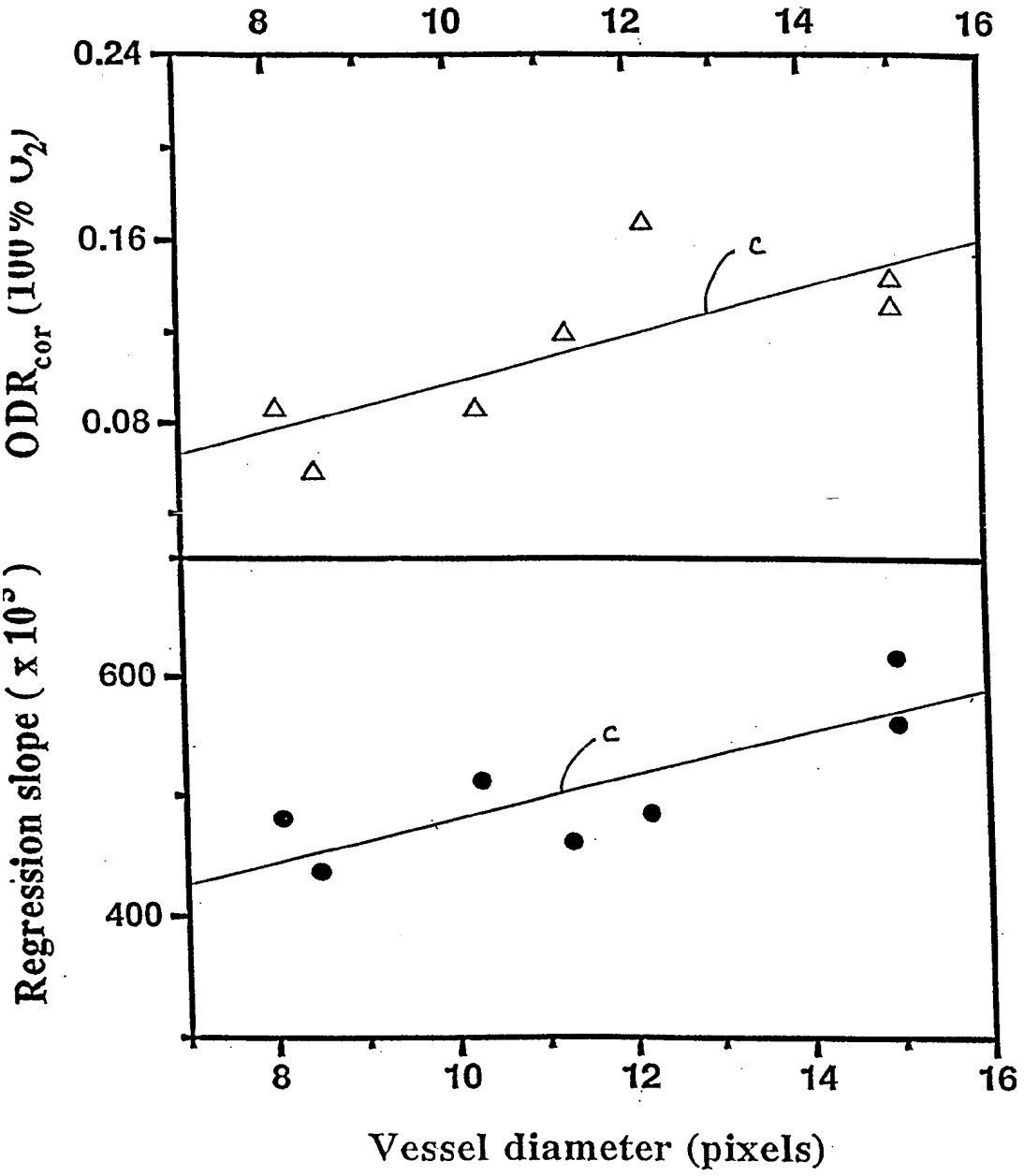
8
10
12
14
16
0.24
0.16
0.08
ODR$_{cor}$ (100% O_2)
c
600
400
Regression slope (x 10^3)
c
8
10
12
14
16
Vessel diameter (pixels)

FIG. 15

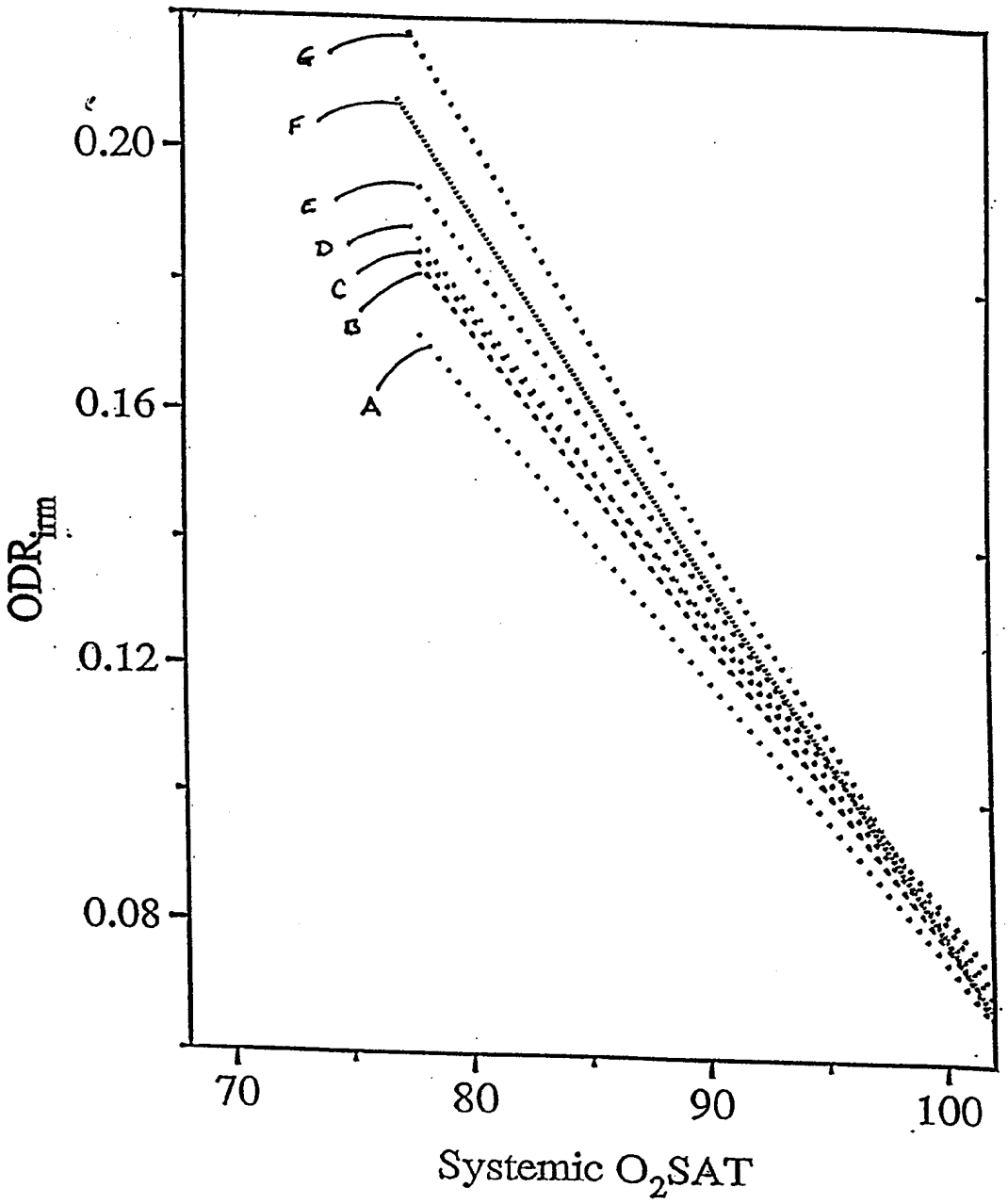

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/17204

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61B 5/00
US CL :600/318, 340

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 600/318, 320, 322, 323, 340,473, 476

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,877,322 A (HILL) 31 October 1989, the entire document.	1-47
A	US 5,240,006 A (FUJII et al) 31 August 1993, entire document.	1-47
A	US 5,308,919 A (MINNICH) 03 May 1994, entire document.	1-47

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 15 NOVEMBER 1999	Date of mailing of the international search report 23 DEC 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ERIC F. WINAKUR Telephone No. (703) 308-3940

Form PCT/ISA/210 (second sheet)(July 1992)*